

Exhibit 99

From: Musco, Nancy [CPCUS]
Sent: Wednesday, May 27, 2009 7:25 PM
To: Chase, David J. (Dr.) [CPCUS]; Martin, Katharine [CPCUS]; Telofski, Lorena [CPCUS]
Subject: FW: Q&A Baby Powder
Attachments: What are the risks of inhalation.doc

Sensitivity: Confidential

All,

Who provided the data from the American Association of Poison Control? This is the information that I spent last week collecting and was putting in table form along with the J&J data. Perhaps I misunderstood my assignment but this represents much duplicate effort.

Thanks,

Nancy

From: Chase, David J. (Dr.) [CPCUS]
Sent: Wednesday, May 27, 2009 1:32 PM
To: Telofski, Lorena [CPCUS]
Cc: Wajszczuk, Charles [CPCUS]; Martin, Katharine [CPCUS]; Musco, Nancy [CPCUS]
Subject: FW: Q&A Baby Powder
Sensitivity: Confidential

Lorena,

Do you know where we could find data of the type I mentioned in my item # 3 (2 e-mails down)? Complete reports would be good, but brief summaries of the reports or statements based on the reports used by the company in the past would probably be more useful acutely.

Thanks,

David

<<What are the risks of inhalation.doc>>

From: Wajszczuk, Charles [CPCUS]
Sent: Wednesday, May 27, 2009 1:16 PM
To: Chase, David J. (Dr.) [CPCUS]; Costabel-Farkas, Margit [CONDE]; Martin, Katharine [CPCUS]
Cc: Ries, Gerd [CONDE]; Giernoth, Judith [CONDE]; Kuijpers, Harold [CONDE]; Andresen, Edda [CONDE]
Subject: RE: Q&A Baby Powder
Sensitivity: Confidential

David and All,

I'll leave determination regarding questions 1 and 2 below to you all. The information referred to in #3 would be of value. For #4, I don't know if anything other than sales data for ALL talc baby powder would be of value. These are numbers for all talc, but I'm guessing (since the info is not available) that most exposures are to baby powder. Chances of having

21 years of sales in the USA might be hard to find. It might be worth stating that the cases with no outcome may represent inquiries, not exposures, but we don't have that info either.

Charlie

Charlie

From: Chase, David J. (Dr.) [CPCUS]
Sent: Wednesday, May 27, 2009 12:59 PM
To: Wajszczuk, Charles [CPCUS]; Costabel-Farkas, Margit [CONDE]; Martin, Katharine [CPCUS]
Cc: Ries, Gerd [CONDE]; Giernoth, Judith [CONDE]; Kuijpers, Harold [CONDE]; Andresen, Edda [CONDE]
Subject: RE: Q&A Baby Powder
Importance: High
Sensitivity: Confidential

This strikes me as being a fairly complete analysis. I took the liberty of making a few suggestions concerning wording.

I also have a few larger questions:

1. Will this document be reviewed by legal, for example, John O'Shaughnessy, who has had a great deal of experience with talc issues over the years?

2. Will it be reviewed by external PR advisors with experience in talc issues?

3. Should it include empirical information on levels of exposure (inhalation) known to be likely from normal use of the product according to instructions, and on the magnitude of those levels compared to amounts of exposure needed to induce cancer or other adverse effects in animal studies? I understand that such information is available and has been made available in previous talc PR cases.

4. Is there any comparison that could/should be made between the AAPCC data and the data reported by the poison centers in Germany, Austria, and Switzerland? If so, should that be included?

David

<< File: What are the risks of inhalation.doc >>

From: Wajszczuk, Charles [CPCUS]
Sent: Wednesday, May 27, 2009 8:17 AM
To: Costabel-Farkas, Margit [CONDE]; Chase, David J. (Dr.) [CPCUS]; Martin, Katharine [CPCUS]
Cc: Ries, Gerd [CONDE]; Giernoth, Judith [CONDE]; Kuijpers, Harold [CONDE]; Andresen, Edda [CONDE]
Subject: RE: Q&A Baby Powder

All, << File: What are the risks of inhalation.doc >> Please see the write up. I think it addresses most of the issues.

Charlie

From: Costabel-Farkas, Margit [CONDE]
Sent: Monday, May 25, 2009 8:57 AM

To: Wajszczuk, Charles [CPCUS]; Chase, David J. (Dr.) [CPCUS]; Martin, Katharine [CPCUS]
Cc: Ries, Gerd [CONDE]; Giernoth, Judith [CONDE]; Kuijpers, Harold [CONDE]; Andresen, Edda [CONDE]
Subject: RE: Q&A Baby Powder
Importance: High

Dear all,

please find attached the draft answers to the questions provided by B-M, prepared by Edda, Judith and me.

Charlie, David, Katharine, could you maybe add any info on consequences or treatment of powder inhalation (two parts highlighted in yellow)?

It would be great to have your input as soon as possible, in order to share the draft with B-M for a final review by tomorrow.

Many thanks and best regards,

Margit

<< File: Baby Powder Questions_QA draft May 22.doc >>

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From: Andresen, Edda [CONDE]
Sent: Freitag, 22. Mai 2009 12:00
To: Costabel-Farkas, Margit [CONDE]
Cc: Ries, Gerd [CONDE]
Subject: Q&A Baby Powder

Hallo Margit,

ich habe angefangen, die Antworten einzufügen. Kannst du die gesundheitlichen Risiken ausführen und über den Rest drüberschauen?
Viele Grüße

Edda

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JNJ TALC000319188

Metadata

Custodian	Martin, Katharine	ORIGINAL
DateCreated	04/03/2010 12:00 AM	ORIGINAL
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FileName	FW: Q&A Baby Powder	ORIGINAL
From	"Musco, Nancy [CPCUS] [/O=JNJ/OU=CPCUSSK/CN=RECIPIENTS/CN=NMUSCO]"	ORIGINAL
ProdVol	TALC_PROD_033	ORIGINAL
Subject	FW: Q&A Baby Powder	ORIGINAL
To	"Chase, David J. (Dr.) [CPCUS];Martin, Katharine [CPCUS];Telofski, Lorena [CPCUS]"	ORIGINAL

Exhibit 100

TALC

1. Chemical and Physical Data

1.1 Synonyms and trade names

CAS Registry No.: 14807-96-6

Chem. Abstr. Name: Talc

Synonyms¹: Soapstone; steatite; talcum

Trade names¹: Agalite; Asbestine; B9 Finntalc P40; B13; B13 (mineral); Beaver White 200; CP 10-40; CP 38-33; Crystalite CR 6002; Desertalc 57; Emtal 500; Emtal 549; Emtal 596; Emtal 599; Fibrene C 400; French Chalk; FW-XO; HSDB 830; IT Extra; LMR 100; Microneeca K1; Micro White 5000A; Microtalc IT Extra; Mistron; MP 25-38; MP 40-27; MP 45-26; MST; MT 12-50; Mussolinite; NCI-CO6018; Nyltal 200; Nyltal 400; Pk-C; Pk-N; Polytal 4641; Polytal 4725; Potstone; Snowgoose; Steawhite; Supreme; Supreme dense; Talcan PK-P; Talcron CP 44-31

1.2 Structure of typical mineral

Molecular formula: $\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$

The original X-ray spectra of talc (Gruner, 1934; Hendricks, 1938) indicated that the mineral had a monoclinic structure. Later investigations (Rayner & Brown, 1966; Ross *et al.*, 1968) demonstrated that many if not all talcs are triclinic (Table 1). The basis of the talc structure is characterized by a hexagonal sheet arrangement of SiO_4 tetrahedral groups linked in a common plane. Each SiO_4 tetrahedron shares three planar oxygen atoms with its neighbouring tetrahedra; the fourth oxygen, the apex of the tetrahedron, is not shared. Two such sheets are orientated so that unshared apical oxygen atoms face each other. The sheets are bonded by magnesium atoms, which are coordinated by two oxygens and one hydroxyl group from each sheet, which form a brucite layer. This structural arrangement results in a double-sheet structure in which the valency demands of the constituent atoms are completely satisfied. Crystals of talc are made up of stacks of these double-sheet units held together by the weakest of chemical bonds — the Van der Waal's forces. As the individual sheets cannot be bonded together, they can be separated by slight forces, causing slippage of the individual sheets along a perfect cleavage direction in the basal plane (Rohl *et al.*, 1976; Pooley & Rowlands, 1977).

¹These synonyms and trade names cover talc, talc-containing materials and talc contaminated with other minerals as admixtures.

Table 1. Lattice parameters and crystallographic axes of talc

Lattice parameters (nm)			Crystallographic axes			System	Reference
a	b	c	α	β	γ		
0.526	0.910	1.881	90°00'	100°00'	90°00'	Monoclinic	Gruner (1934)
0.527	0.913	1.888	90°00'	100°15'	90°00'	Monoclinic	Hendricks (1938)
0.528	0.915	1.89	90°00'	100°15'	90°00'	Monoclinic	Roberts <i>et al.</i> (1974)
0.5255	0.9137	0.9448	90°46'	98°55'	90°00'	Triclinic	Ross <i>et al.</i> (1968)
0.5293	0.9179	0.9496	90°57'	98°91'	90°03'	Triclinic	Ross (1984)

1.3 Chemical and physical properties

From Roberts *et al.* (1974)

- (a) *Hardness*: 1 on Mohs' scale
- (b) *Density*: 2.58-2.83
- (c) *Cleavage*: (001) perfect
- (d) *Colour*: Pale-green to dark-green or greenish-grey; also white, silvery-white, grey, brownish; translucent; pearly, greasy or dull
- (e) *Description*: Commonly thin tabular crystals, up to 1 cm in width. Usually massive, fine-grained, compact; also as foliated or fibrous masses or in globular stellate groups

1.4 Technical products and impurities

The chemistry of talc shows little variation, indicating that only a limited substitution of ions takes place in the mineral lattice. When expressed in the standard oxide form, the ideal chemical composition is: 31.7% MgO, 63.5% SiO₂, 4.8% H₂O (Pooley & Rowlands, 1977). Small amounts of aluminium and titanium may substitute to some extent for silicon, and it is common to find iron, nickel, manganese or chromium substituting to some extent for magnesium. Iron and nickel substitute for magnesium in the greatest amounts (Pooley & Rowlands, 1977), and a talc with almost complete substitution of magnesium by iron, called minnesotaite, is abundant in the iron formations of Minnesota, USA (Deer *et al.*, 1971). One major talc deposit in the eastern USA contains substantial amounts of nickel — up to 0.2% (Rohl *et al.*, 1976). Nickel-substituted talcs are also associated with serpentine bodies, at up to 0.5% by weight (Pooley & Rowlands, 1977). Table 2 gives examples of the mineral composition of talcs (Deer *et al.*, 1971).

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Table 2. Bulk chemical analysis of talcs (%)^a

Component	Talc ^b								
	1	2	3	4	5	6	7	8	9
SiO ₂	62.61	62.67	62.47	62.16	60.06	60.02	60.88	61.07	51.29
TiO ₂	—	—	—	—	—	—	0.10	—	0.04
Al ₂ O ₃	—	0.38	0.47	0.88	1.60	1.88	1.98	2.42	0.61
Fe ₂ O ₃	—	0.68	—	—	—	—	0.83	1.49	2.00
FeO	2.46	0.65	0.79	1.41	1.74	1.51	—	—	33.66
MnO	0.01	—	0.00	—	—	—	—	—	0.12
MgO	30.22	29.95	31.76	30.86	30.83	30.39	31.18	29.13	6.26
CaO	—	1.35	0.00	—	0.40	1.00	0.14	0.75	0.00
Na ₂ O	—	—	—	—	—	—	—	—	0.08
K ₂ O	—	—	—	—	—	—	—	—	0.03
H ₂ O ⁺	4.72	5.05	4.70	4.92	5.02	5.37	4.98	4.82	5.54
H ₂ O ⁻	—	—	0.06	—	—	0.32	—	—	0.24

^aFrom Deer *et al.* (1971)

^b1, talc, altered periodotite, Muruhatten, northern Sweden; 2, talc, Shabrov, Urals, USSR; 3, talc, Murphy, North Carolina, USA; 4, light-green talc, Malangen, Norway; 5, green talc, altered serpentine, Parma district, Appenines, Italy; 6, black talc, with carbonaceous material derived from a bluish-grey rock, Parma, Appenines, Italy; 7, talc, Mount Fitton, South Australia; 8, talc, altered tremolite, Yellandu Warangal district, Hyderabad, India; 9, greenish-grey iron talc (minnesotaite), East Mesabi range, Minnesota, USA

Since talc is formed by alteration or metamorphosis of rocks, it is found associated with many types of minerals. Rohl *et al.* (1976) listed the following minerals as commonly occurring in talc deposits: calcite, dolomite, magnesite, tremolite, anthophyllite, antigorite, quartz, pyrophyllite, micas and chlorites. Chrysotile and lizardite were noted as 'uncommon' constituents. When mined, talc ore may contain several of the minerals noted in Table 3.

In one study of Vermont (USA) talc, the mined and milled ore contained 20-100% each of talc and magnesite, a small amount of chlorite (5-20%) and minor amounts (<5%) of dolomite, calcite, quartz, phlogopite and biotite (Boundy *et al.*, 1979). An analysis of samples of mined and milled talc from New York (USA) yielded the following concentrations of minerals: talc, 12-50%; tremolite, 30-55%; anthophyllite, 3-35%; serpentine, 1-8%; calcite, <1-4%; and quartz, <0.1-20% (Schepers & Durkan, 1955a). A more recent examination of talc from Texas (USA) showed the presence of fibrous tremolite and antigorite (Gamble *et al.*, 1982). Rohl *et al.* (1976) showed that some US talcum powders marketed prior to 1975 contained chlorite, phlogopite, calcite, dolomite, quartz, kaolin, tremolite, anthophyllite, chrysotile, pyrophyllite and rutile. One French talc (Luzenac 15MOO) has been reported to contain 90% talc, 8% chlorite, 1% dolomite and no asbestos fibre (Talc de Luzenac, 1982). An Italian talc (grade 00000) was reported to contain 92% talc, 3% chlorite, 1% carbonates and 0.5-1% quartz and no tremolite or chrysotile asbestos (Wagner *et al.*, 1977).

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Table 3. Minerals that occur commonly in talcs^a

Mineral group	Phase	Formula
Carbonates	Calcite	CaCO_3
	Dolomite	$\text{CaMg}(\text{CO}_3)_2$
	Magnesite	MgCO_3
Amphiboles	Tremolite ^b	$\text{Ca}_2\text{Mg}_5\text{Si}_8\text{O}_{22}(\text{OH})_2$
	Anthophyllite ^b	$(\text{FeMg})_7\text{Si}_8\text{O}_{22}(\text{OH})_2$
Serpentine	Antigorite	$\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4$
	Chrysotile (uncommon)	$\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4$
	Lizardite (uncommon)	$\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4$
Others	Quartz	SiO_2
	Mica, e.g., phlogopite	$\text{K}_2(\text{Mg,Fe})_6[\text{Si}_6\text{Al}_2\text{O}_{20}](\text{OH})_4$
	Chlorite, e.g., penninite	$(\text{Mg,Al,Fe})_{12}[(\text{Si,Al})_8\text{O}_{20}](\text{OH})_{16}$
	Pyrophyllite	$\text{Al}_4[\text{Si}_8\text{O}_{20}](\text{OH})_4$

^aFrom Rohl *et al.* (1976)^bOccurring as nonasbestiform and asbestiform varieties

Technical products of talc are sold in a multitude of grades, which have functional or physical characteristics especially suited for certain applications. Clifton (1985) outlined the following guidelines for talc specifications by end use:

Ceramics: Uniform chemical and physical properties are required. Manganese and iron are usually objectionable. For high frequency insulators, no more than 0.5% calcium oxide, 1.5% iron oxide and 4% aluminium oxide can be tolerated.

Paints: Impurities that grind to colours other than white are highly objectionable. To yield the desired smooth paint film, at least 98.5% must pass through a 325-mesh screen.

Roofing: A low-grade, off-colour, impure talc is acceptable.

Insecticides: Requirements are chemical inertness with respect to toxicants, satisfactory bulk density and low abrasive characteristics.

Rubber: Many synthetic rubbers include ground talc as fillers in compounding formulations.

Cosmetics and pharmaceuticals: Talc must be grit free, finely sized, chemically pure and pleasing in colour. For cosmetics, talc must have good dry-slip characteristics.

Paper: Requirements include chemical inertness, softness, freedom from grit, satisfactory ink acceptance, brightness and dispersibility in water.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(a) Production

Talc-containing rocks were first used in prehistoric times for utensils and ornaments (Roe & Olson, 1983); the term 'talc' was first applied to this mineral in 869 AD (Kužvart, 1984). The abundance of talc and the facility with which it can be mined, combined with its many desirable functional properties, have made it an important industrial mineral. Mining of talc for commercial purposes probably began several hundred years ago when talc blocks were used for building materials and cooking utensils (Clifton, 1985).

The world reserve base of talc and the related aluminium silicate, pyrophyllite, is estimated to be 1200 million tonnes (Clifton, 1985; Table 4).

Table 4. Worldwide reserve base of talc and pyrophyllite^a

Region	Million tonnes
Africa	18
North America	580
South America	18
Asia and Oceania	362
Europe	172

^aFrom Clifton (1985); talc and pyrophyllite are not distinguished.

The first talc-grinding mill in the USA began operation in about 1880, suggesting the first large-scale US production of ground talc products. US production for many years continued to include both ground talc products and carved items (Clifton, 1985). 'Soapstone' blocks were first produced in open-pit operations, and the vast majority of world talc mining operations continue to rely on open-cast mining methods. Notable exceptions are in Austria and Italy, where necessity or the prospect of high-grade talc in deeper deposits has made underground mining an economically viable operation (Clarke, 1979). Talc sold in blocks is generally removed using hand tools; talc for grinding is mined by drilling and blasting methods (Clifton, 1985).

Practices for refining talc ores vary widely. In some operations, such as those of one mine in France, talc is initially sorted by hand to supply cosmetic talcs of different colour and physical characteristics (Clarke, 1979). Since most uses of talc have not required highly pure products, beneficiation and sophisticated milling and other processing techniques have not been used before shipping. Early talc mills were used to process both talc and cereal grains, the final product in both cases being a coarse powder (Roe & Olson, 1983).

The latest technology in talc refining employs flotation separation, drying of the filtered powder cake, and sizing or further grinding before shipping (Roe & Olson, 1983; Clifton, 1985). Flotation techniques are especially prevalent in North American, Norwegian and Finnish operations (Sinha, 1982).

Talc is mined in over 40 countries and is used in numerous manufacturing industries in over 60 countries (Roe & Olson, 1983; Harben & Bates, 1984). Commercial talc production figures in 1950-1983 are listed by region in Table 5.

Table 5. Talc production by world region, 1950-1983 (1000 tonnes)^a

Region/ country	Important producers	Year						
		1950	1960	1970	1980	1981	1982	1983
Africa	Egypt, South Africa	8	9	14	14	11	18	10
Asia	China, Republic of Korea	7	181	354	1312	1280	1248	1290
Australia		9	16	48	160	75	143	150
Europe	France, Italy, Austria, Finland, Norway	344	553	820	1180	1153	1195	1140
India and Pakistan		25	95	161	379	380	348	292
Japan		12	50	138	148	120	106	85
North America	USA	475	585	891	1124	1218	1035	998
South America	Brazil, Argentina	13	50	118	380	376	354	371
USSR		—	250	380	490	500	510	510

^aFrom Colonial Geological Surveys (1957); Institute of Geological Sciences (1967, 1978); British Geological Survey (1985). Figures are given for 'talc', although sometimes figures were not provided separately for talc production and pyrophyllite production.

Although the largest producers of talc are typically net exporters, notably Australia, Austria, China, France and the USA, several import talc in large quantities as well. Japan is by far the most important world market for talc, importing over 615 200 tonnes in 1983. Canada, the Federal Republic of Germany, Mexico, the UK, the USA and the USSR account for most of the remainder of talc imports (British Geological Survey, 1985).

(b) Use

Talc is one of the most versatile inorganic substances available to industry (Roe & Olson, 1983). Since its uses are dependent on the mineral character of the refined ore, more than many other industrial minerals, talc ores are often referred to by physical type, and used according to their functional characteristics. Although use patterns vary substantially from region to region, four major applications may be highlighted.

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Ceramics

In the USA, 35%, and in Europe, nearly 10%, of native or imported talcs is used in ceramics (Anon., 1982; Clifton, 1985). Talc is especially useful in ceramics for its colour, fast-firing and low shrinkage properties. It has been used in floor- and wall-tiles, china, glazes, electrical porcelains, sanitary ware, kiln furniture and pottery (Clifton, 1985). Some china contains 15% talc by weight, while some pottery contains 40%. Up to 80% talc has been used in ceramic insulators (Roe & Olson, 1983).

Paper

The most important use of talc in Europe and Japan and the fastest-growing use in the USA is in the coating and filling of paper (Anon., 1982; Roe & Olson, 1983). The largest talc importer, Japan, uses 80% of its imports in the paper industry (Clarke, 1979). This statistic, combined with the 50% consumption pattern of talc for paper in Europe (Anon., 1982), makes this the predominant use of talc in the world (Clarke, 1979).

Plastics and building materials

In 1983, about 165 000 tonnes of talc were used in the plastics, rubber and roofing industries in the USA, representing 20% of the total consumption (Clifton, 1985). Talc has become an important component of many types of US plastics, as a stabilizer, reinforcer and filler used at up to 70% w/w. In roofing materials, talc is added at 10-35% to asphalt in composite shingling materials, to impart stability and weather resistance (Roe & Olson, 1983).

Paints

Approximately 15-25% of the talc used in most industrialized nations is as a pigment extender and filler in paints (Anon., 1982). As with the paper application, fineness of grade and colour are most important to the functional characteristics of the compound. US consumption of talc for use in paints was 213 000 tonnes in 1979 (Roe & Olson, 1983) and 150 000 tonnes in 1983 (Clifton, 1985).

Other uses

A significant, although less commercially important use of talc is in cosmetics. In the USA and Europe, approximately 5% of native and imported ores are used for this purpose (Anon., 1982; Clifton, 1985). Talc is directly available to consumers as facial cosmetics and talcum powders. US talcum powders marked prior to 1973 contained up to 95% by weight talc mineral; however, some commercial talcum powders contained no talc (e.g., starch was used). Mineral impurities such as amphibole minerals (tremolite, anthophyllite) and quartz were found in concentrations up to 14 and 35% by weight, respectively (Rohl *et al.*, 1976). Talc is also used as an excipient in pharmaceuticals and as a filler in toothpastes and soaps (Rohl & Langer, 1979; Kuřvart, 1984).

Other uses of talc are as a cereal grain polisher (especially rice), as an ingredient in floor waxes and shoe polishes, as a carrier and diluent for pesticides, as a textile component, as an oil absorber, as a lubricant and in spackling and patching compounds (Rohl & Langer, 1979; Roe & Olson, 1983; Clifton, 1985).

(c) *Regulatory status and guidelines*

Occupational exposure limits in various countries are listed in Table 6.

Table 6. Occupational exposure limits for talc (mg/m³)^a

Country	Year	Total dust (mg/m ³)	Respirable dust (mg/m ³)
Australia	1978	2.5	
Czechoslovakia	1976	6	
Finland	1981	5 ^b	
France	1985		2 ^b
Italy	1978	5	1.6
Norway	1981	6	
United Kingdom	1985	10	1
USA			
ACGIH	1986		2 ^b
OSHA	1983	(20 mppcf) ^{b,c}	
USSR	1976	4	
Yugoslavia	1971	12	4

^aFrom International Labour Office (1980); Direktoratet för Arbeidstilsynet (1981); Työsuojeluhallitus (1981); US Occupational Safety and Health Administration (OSHA) (1983); Health and Safety Executive (1985); Institut National de Recherche et de Sécurité (1985); American Conference of Governmental Industrial Hygienists (ACGIH) (1986)

^bAsbestos fibre standards are used for fibrous forms

^cContaining <1% quartz

2.2 Occurrence

(a) *Natural occurrence*

Talc rocks are formed by several complex geological processes reacting upon many chemically diverse preexisting rock types. Hydrothermal alteration of magnesia- and silica-rich ultramafic rocks, under a range of low-to-moderate temperatures and pressures, may produce talc. Thermal metamorphosis of silica-rich dolomite will also produce talc. These processes, however, also commonly result in the formation of a number of other coexisting mineral phases — predominantly hydrous magnesium silicates. Some of these — for example, anthophyllite, tremolite and serpentine minerals (including chrysotile) — may occur as microscopic intergrowths with talc, as macroscopic nodules, or even as discrete zones within or adjacent to talc. Talc rock is therefore often a mixture of minerals varying in kind and quantity (Rohl & Langer, 1974; Rohl *et al.*, 1976; Clifton, 1985).

Fibre intergrowths are often such that even extensive beneficiation may not yield a pure product. Thus, where fine-grained intergrowths of talc and tremolite occur, the processed product will probably contain residual tremolite (Rohl *et al.*, 1976).

(b) *Occupational exposure*

Talc-milling processes do not usually alter the mineral composition of the talc mixture delivered to the mill, but rather produce a talc with different physical properties dependent on particle size. Exposure to talc dust occurs during mining, crushing, separating, bagging, loading and in end-use facilities, such as rubber dusting and addition of talcs to ceramic clays and glazes. Since industrial talc is a mixture of various associated minerals, occupational exposure is to a mixture of mineral dusts.

Studies that provide information on occupational exposures to talc are summarized in Table 7 and described in more detail below. As with most industrial dust exposures, nearly all measurements made prior to approximately 1970 were done by collecting particles in an impinger and counting them by optical microscopy. Concentrations are thus expressed as millions of particles per cubic foot of air (mppcf).

In Georgia, USA, average dust exposures for miners using jackhammer drills were 1440 mppcf and those for millers 52 mppcf. The talc was reported to contain 45% tremolite and 45% talc, with little or no free silica (Dreessen, 1933). Average dust concentrations in a talc mine were reported to range from 32-855 mppcf (six samples), whereas average mill exposures ranged from 17-1672 mppcf (14 samples). The dust was reported to contain 70% talc, 20-30% dolomite and 10% tremolite, and no free silica except for occasional fragments; its morphology was described as 'bladed crystals'. Highest dust exposures were in bagging operations (Dreessen & DallaValle, 1935).

Occupational exposures to talc dust in mines and mills in New York State, USA, have been studied extensively (Siegal *et al.*, 1943; Kleinfeld *et al.*, 1955; Messite *et al.*, 1959; Kleinfeld *et al.*, 1967, 1974; Dement & Zumwalde, 1979; Dement *et al.*, 1980). Talc deposits in the state have been found to differ significantly in mineral composition, depending on location. Siegal *et al.* (1943) reported that talc produced in St Lawrence County contained tremolite, anthophyllite and only traces of quartz, and described the particle morphology as straight, needle-like fibres with a maximum length of 15 μm . Kleinfeld *et al.* (1973) also reported the major fibrous components of these talcs to be tremolite and anthophyllite, based on detailed electron microscopic observations. Bulk talc samples from another mine and mill in upper New York State were analysed for mineral content by optical petrographic microscopy, electron microscopy and X-ray diffraction. The mineral composition (by weight) of the talc bulk samples was 14-48% talc, 37-59% tremolite (including both fibrous and nonfibrous habits), 4.5-15% anthophyllite (including both fibrous and nonfibrous habits), 0.25-2.6% free silica, 0.0-1% calcite, 0.5-1% dolomite and 10-15% serpentines (largely lizardite and antigorite) (Dement & Zumwalde, 1979; Dement *et al.*, 1980).

Talc dust and fibre exposures in mining and milling operations in St Lawrence County, NY, for the period 1945-1972 are summarized in Table 8. Prior to dust control measures, such as wet drilling, average exposures to mine dust ranged from 120-818 mppcf; after 1945, these were reduced to 5-19 mppcf. Exposures in mills prior to 1945 ranged from 69-278 mppcf; average exposures in 1972 ranged from 7-36 mppcf. In 1972, optical fibre counts, using membrane-filter sampling and analyses, revealed that exposures in mines were low

Table 7. Studies of occupational exposures to talc

Reference	Industry studied	Location of talc deposit	Date of exposure measurements	Measurement method employed	Other minerals present in talc studied
Dreessen (1933)	Mining/Milling	Georgia, USA	Pre 1933	Impinger	Tremolite
Dreessen & DallaValle (1935)	Mining/Milling	Georgia, USA	Pre 1935	Impinger	Tremolite, dolomite
Siegal <i>et al.</i> (1943)	Mining	New York, USA	1940-1941	Impinger	Tremolite, anthophyllite, traces of free silica
Kleinfield <i>et al.</i> (1955); Messite <i>et al.</i> (1959); Kleinfield <i>et al.</i> (1967, 1974)	Mining/Milling	New York, USA	Pre 1945-1972	Impinger	Tremolite, anthophyllite, carbonates, traces of free silica
Kleinfield <i>et al.</i> (1973)	Mining/Milling	New York, USA	1954-1970	Impinger, optical fibre counts	Tremolite, anthophyllite
Dement & Zumwalde (1979); Dement <i>et al.</i> (1980)	Mining/Milling	New York, USA	1975	Gravimetric, optical and electron microscopy fibre counts	Tremolite, calcite, anthophyllite, dolomite, serpentines, silica
Rubino <i>et al.</i> (1976)	Mining/Milling	Piedmont, Italy	1920-1975	Impinger	Small amounts of tremolite
Boundy <i>et al.</i> (1979)	Mining/Milling	Vermont, USA	1975-1976	Optical and electron microscopy fibre counts	Dolomite, calcite, magnesite, chlorite, traces of other minerals
Greife (1980); Gamble <i>et al.</i> (1982)	Mining/Milling	Montana, Texas and North Carolina, USA	1977-1980	Gravimetric	Varied by location studied
Hogue & Mallette (1949)	Rubber dusting	Vermont, USA	1943-1948	Impinger	Stated to be 'pure talc'
Dement & Shuler (1972)	Rubber dusting	Not stated (USA)	1972	Gravimetric, optical fibre counts	2-3% free silica
Fine <i>et al.</i> (1976)	Rubber dusting	Vermont, USA	1972-1974	Gravimetric	Trace of silica (<1%), <2 fibres/cm ³

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Table 8. Average dust and fibre concentrations in St Lawrence County, NY, talc mining and milling operations, pre-1945-1972^a

Exposure	Dust exposure (mppcf)				Fibres ^b
	Before 1945	1946-1965	1966-1969	1972	1972
<i>Mining</i>					
Drilling	818	5	19	7	3
Mucking	120	5	9	3	2
<i>Milling</i>					
Crushing	180	42	28	35	62
Screening	69	37	—	—	—
Milling	92	25	40	7	25
Garnering and separating	278	27	—	13	27
Pulverizing	—	28	—	—	—
Bagging	151	27	29	27	47
Box car and lorry loading	—	73	43	36	24

^aFrom Kleinfeld *et al.* (1974)^bNumber of fibres/cm³ >5 µm in length (by phase-contrast microscopy)

(2-3 fibres >5 µm/cm³), whereas exposures in mills ranged from 25-62 fibres/cm³ (Kleinfeld *et al.*, 1974). [The Working Group noted that the fibre counts represent optical counts of all fibres with a 3:1 aspect ratio and longer than 5 µm, with no further mineral identification.]

Data on time-weighted-average exposures to respirable dust and airborne fibres in the mine and mill studied by Dement *et al.* are shown in Table 9. Time-weighted average exposures to respirable dust ranged from 0.23-1.29 mg/m³ in the mine and 0.25-2.95 mg/m³ in the mill. Due to the low free silica content of this talc, exposure to respirable free silica did not exceed 0.025 mg/m³ in the mine and 0.028 mg/m³ in the mill. Airborne fibre levels measured by optical microscopy gave mean exposures in the mine and mill of 4.5 and 5.0 fibres >5 µm/cm³, respectively, with peak values as high as 29.1 fibres/cm³ in the mill. Further analyses of the airborne fibre samples by electron microscopy showed that 65% of the fibres greater than 5 µm in length were anthophyllite and 7% were tremolite. The authors concluded that the most important fibrous component of this talc deposit was anthophyllite (Dement & Zumwalde, 1979; Dement *et al.*, 1980).

Concentrations of respirable dust in mass samples from three Vermont talc mines and mills surveyed in 1975-1976 are given in Table 10. Geometric mean exposures to respirable dust ranged from 0.5 to 5.1 mg/m³ in the mines and from 0.5 to 2.9 mg/m³ in the mills; however, exposures in the mills were generally higher than those in the mines. Optical fibre counts of as much as 60 fibres/cm³ were reported. Subsequent analyses of these samples by scanning electron microscopy demonstrated rolled talc and elongated talc particles. X-ray diffraction analyses of bulk samples from these mines and mills showed that talc and magnesite were the major (20-100%) mineral components, chlorite and dolomite minor (5-20%) components, and that dolomite, calcite, quartz, biotite, ankerite, chromite,

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Table 9. Respirable dust exposures and airborne fibre (longer than 5 μm) concentrations^a in a New York state talc mine and mill^b

Operation	Respirable dust				Airborne fibres				
	No. of samples	Time-weighted average ^c		Highest peak ^d (mg/m ³)	No. of samples	Time-weighted average ^c			Highest peak ^d (fibres >5 $\mu\text{m}/\text{cm}^3$)
		Mean	Range			Mean	Median	Range	
Mine	14	0.86	0.23-1.29	1.72	54	4.5	4.4	0.8-9.8	18.2
Mill	29	0.86	0.25-2.95	4.64	168	5.0	4.3	0.2-16.0	29.1

^aBy optical microscopy^bFrom Dement and Zumwalde (1979)^cFull shift determinations^dBased on highest concentration observed in a single sample**Table 10. Respirable dust concentrations (mg/m³) in Vermont talc mines and mills^a**

Company	Area	Summer 1975		Winter 1976	
		No. of samples	Geometric mean (mg/m ³)	No. of samples	Geometric mean (mg/m ³)
A	Underground mine	18	0.6	16	0.5
	Mill (1st shift)	4	1.7	13	1.7
	Mill (2nd shift)	6	0.5	3	1.5
B	Underground mine	15	1.5	23	0.9
	Mill (1st shift)	22	1.8	42	1.8
	Mill (2nd shift)	12	2.9	16	1.9
C	Underground mine	12	0.5	19	0.7
	Walk-in mine	7	1.2		
	Walk-in mine			6	1.7
	Open-pit mine	2	5.1	—	—
	Mill # 1 (1st shift)	12	0.9	20	1.1
	Mill # 1 (3rd shift)	3	0.8	4	1.4
	Mill # 2 (1st shift)	11	1.0	8	0.5
	Mill # 2 (2nd shift)	13	0.8	3	1.1

^aFrom Boundy *et al.* (1979)

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phlogopite and oligoclase were present in smaller amounts (<5%). Trace amounts of free silica were found in 15% of the samples (Boundy *et al.*, 1979). One closed mine was reported to contain tremolite microinclusions, but its fibrosity was not documented (Selevan *et al.*, 1979).

A cross-sectional study of occupational exposures in US talc mines and mills was conducted by the National Institute for Occupational Safety and Health; the results are summarized in Table 11. Bulk samples from each region were analysed by transmission electron microscopy: no fibre was found in any sample of Montana talc; fibrous tremolite and antigorite were reported in Texan talcs (0.5-3.0 μm in diameter, 4-30 μm in length); and talcs from North Carolina contained acicular cleavage fragments with particle length: diameter ratios as high as 100:1, with some <0.1 μm in diameter (Greife, 1980; Gamble *et al.*, 1982).

Table 11. Respirable dust concentrations in 275 samples from talc mines and mills located in Montana, Texas and North Carolina, USA^a

Samples	Geometric mean (mg/m ³)		
	Montana	Texas	North Carolina
From mines	0.66 (0.47-0.92) ^b	0.45 (0.18-0.71)	0.14 (0.07-0.31)
From mills	1.1 (0.85-1.41)	1.56 (0.96-2.54)	0.26 (0.13-0.51)
Bulk talc samples (% free silica)	<0.8	2.23	1.45

^aAdapted from Greife (1980) and Gamble *et al.* (1982)

^bIn parentheses, 95% frequency interval

Analysis of 362 personal samples of respirable dust collected over a full shift by the Mine Safety and Health Administration from talc mines and mills in the USA showed the median dust exposure to be 1.20 mg/m³; 90% of all exposures were to less than 2.78 mg/m³ (National Institute for Occupational Safety and Health, 1979).

Prior to adoption of technical preventive means in 1950, exposures in the talc operation in the Germanasca and Chisone Valley (Piedmont), Italy, were reported to be to approximately 800 mppcf in the mines and to 25 mppcf in the mills. Exposures in both areas were reduced to less than 10 mppcf after 1965. Mineralogical analyses of these talcs demonstrated that they contained quartz, muscovite, chlorite, garnet, calcite, magnesite and small quantities of other minerals. In a few specimens, a small amount of tremolite was detected, but no other type of amphibolic asbestos or chrysotile was reported. The free silica content of powdered talc specimens was generally below the detection limits of X-ray diffraction (Rubino *et al.*, 1976). [The Working Group noted that the analytical methods were not described in detail, and the relative fibrosity of the tremolite was not documented.]

Only limited information is available about exposures in secondary industries in which talc is used or processed further. Personal air samples collected in a rubber band production plant, where housekeeping, ventilation and work practices were poor and in which talc was used as an antistick agent, had time-weighted average respirable dust concentrations of 2.5-7.8 mg/m³ (average, 4.8 mg/m³) for extruders, 5.3 and 6.1 mg/m³ for vulcanizers and 0.9 and 1.3 mg/m³ for cutters. Total dust exposures were found to range from 5.4-199 mg/m³. The talc was reported to contain 2-3% free silica. Fibre exposures, as measured by phase-contrast optical microscopy, ranged from 4.7-19.2 fibres >5 µm/cm³ (Dement & Shuler, 1972). [The Working Group noted that no electron microscopic analysis was conducted to confirm the identity of the fibres; however, most of the fibres were probably not asbestos.]

Respirable dust concentrations in two rubber manufacturing plants where Vermont talc was used as an antistick agent are shown in Table 12. Eighteen of 21 samples analysed for free silica contained less than 1% by weight. In 12 samples analysed for fibres, using optical microscopic techniques for asbestos, all concentrations were less than 2 fibres >5 µm/cm³. No electron microscopic fibre analysis was reported (Fine *et al.*, 1976). Hogue and Mallette (1949) found an average dust concentration of 15-50 mppcf talc in two rubber plants using Vermont talc. Tube machine operators had an average exposure of 20 mppcf; tube 'bookers', 35 mppcf; tube cure men, 15 mppcf; and 'line rerollers', 50 mppcf.

Table 12. Respirable dust concentrations in rubber processing plants using talc^a

Location	No. of samples	Average dust concentration (mg/m ³)
<i>Plant A</i>		
Lorry and bus inner tubes (splicer)	7	0.60
Lorry and bus inner tubes (cureman)	6	1.41
'Tuber operator'	3	0.47
'Booker'	3	0.74
Farm service inner tubes (splicer)	6	0.82
Farm service inner tubes (cureman)	2	0.91
<i>Plant B</i>		
Rubber band area	6	3.55
Gum engraving room	6	0.64
Hose extruding	4	0.51
Curing heavy duty flaps	3	1.29
'Dust room'	2	0.59

^aFrom Fine *et al.* (1976)

2.3 Analysis

Because talc is frequently contaminated with a number of other mineral phases, some known to be biologically active, an analytical protocol is often required that can distinguish among these phases.

Phase-contrast optical microscopy is a conventional technique for the identification of minerals. A microscope equipped with bright-field illumination and polarized light optics may be used to analyse talc powders (Hamer *et al.*, 1976; Boundy *et al.*, 1979; Rohl & Langer, 1979). The limitations of the technique for this purpose are discussed by Rohl *et al.* (1976).

The characteristic lines of X-ray powder diffraction pattern are 0.934, 0.468, 0.456, 0.343, 0.3115, 0.2632 and 0.2598 nm (Ross, 1984). Quantitative mineralogical analyses of bulk samples are sensitive to about 1-2% of talc (Pooley & Rowlands, 1977). The application of X-ray diffraction analysis, both continuous and step-scan modes, for quantitative determination of contaminating minerals in talc has been described, including the selection of talc and reference materials, the preparation of standard dilutions of fibres in talc to ensure sensitivity and reproducibility, the selection of characteristic X-ray reflections to be scanned, and instrumental technique. Tremolite, chrysotile and anthophyllite impurities in talc can be determined at levels as low as 0.1-2% (Rohl & Langer, 1974; Rohl *et al.*, 1976).

Morphological, structural and chemical information on single particles of talc and associated minerals can be obtained by analytical electron microscopy and selected-area electron diffraction (Rohl *et al.*, 1976).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals¹

The Working Group noted that in most of the studies of 'talc' described below, no or limited characterization of the mineralogy of the sample employed was given, and, in particular, there was a lack of information on fibre content or particle size.

(a) Oral administration

Rat: Groups of 25 male and 25 female Wistar rats, ten weeks of age, received about 50 mg/kg bw per day commercial talc [characteristics unspecified] in the diet or standard diet for life (average survival, 649 days). No significant difference in tumour incidence was found in comparison with controls (Gibel *et al.*, 1976).

A group of 16 male and 16 female Wistar-derived rats, 21-26 weeks of age, were exposed to 100 mg Italian talc (grade 00000; ready milled; mean particle size, 25 μ m; containing

¹The Working Group was aware of studies in progress in mice and rats by inhalation (IARC, 1986) and in rats by subcutaneous and intraperitoneal injection (Maltoni *et al.*, 1982).

92% talc, 3% chlorite, 1% carbonate minerals and 0.5-1% quartz) per day per rat in the diet for five months and then maintained on basal diet for life (average survival, 614 days). A control group of 16 rats was fed basal diet. No difference in tumour incidence was found between the two groups (Wagner *et al.*, 1977). [The Working Group noted the limited exposure period and the advanced age of the animals at the start of exposure.]

(b) *Inhalation exposure*

Rat: A group of 24 male and 24 female Wistar-derived rats, six to eight weeks of age, was exposed by inhalation to a mean respirable dust concentration of 10.8 mg/m³ Italian talc (grade 00000; ready milled; mean particle size, 25 µm; containing 92% talc, 3% chlorite, 1% carbonate minerals and 0.5-1% quartz) for 7.5 h per day on five days a week for six (24 rats) or 12 (24 rats) months (cumulative exposures, 8200 and 16 400 mg/m³ × h, respectively). Ten days after the end of each exposure period, six rats in each group were killed; a further four rats were killed in each group one year later. Within 28 months of the start of the study, a further 12 animals in each group had died. No lung tumour was observed in rats exposed to talc for six months, while one lung adenoma occurred among those exposed for 12 months. No lung tumour was found in 24 male or 24 female controls (Wagner *et al.*, 1977). [The Working Group noted the limited number of animals allowed to survive longer than 12 months after the end of each exposure period.]

Hamster: Three groups of 50 male and 50 female Syrian golden hamsters, four weeks old, were exposed to an aerosol of talc baby powder, prepared from Vermont talc by flotation (95% w/w platy talc with trace quantities of magnesite, dolomite, chlorite and rutile), for 3, 30 or 150 min per day on five days a week for 30 days. The mean total aerosol concentration was 37.1 mg/m³, with a mean respirable fraction of 9.8 mg/m³ and a mass median aerodynamic diameter of 4.9 µm. Two further groups of hamsters, seven weeks old, were exposed to talc aerosol for 30 or 150 min per day for 300 days or until death. The mean total aerosol concentration was 27.4 mg/m³, with a mean respirable fraction of 8.1 mg/m³ and a mass median aerodynamic diameter of 6 µm. Two control groups of 25 males and 25 females were sham exposed. No primary neoplasm was found in the respiratory system of any hamster. The incidence of alveolar-cell hyperplasia was 25% in the groups exposed to aerosol for 30 or 150 min per day for 300 days, compared with 10% in the control group (Wehner *et al.*, 1977a, 1979). [The Working Group noted the inadequate duration of the study.]

(c) *Intratracheal administration*

Hamster: Groups of 24 male and 24 female Syrian golden hamsters, nine weeks old, received 18 weekly intratracheal injections of 3 mg talc (United States Pharmacopeia grade; 93.3% below 25 µm) in 0.2 ml saline, with or without 3 mg benzo[*a*]pyrene, or 0.2 ml saline only, or were untreated. The animals were allowed to live out their lifespan (average 50% survival, 46-55 weeks). No respiratory-tract tumour was observed in animals exposed to talc alone or in saline-treated or untreated controls. In hamsters exposed to talc with benzo[*a*]pyrene, 33/45 animals had benign and malignant tumours of the respiratory tract

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(larynx to lung) (Stenbäck & Rowland, 1978). [The Working Group noted that no group received benzo[a]pyrene alone and that the survival in all groups was relatively short.]

(d) *Subcutaneous administration*

Mouse: Fifty female R3 mice, three to six months of age, were given single subcutaneous injections of 0.2 ml of a mixture of 8 g talc [unspecified] and 20 g peanut oil [dose, about 80 mg] and observed for life (average 50% survival, 596 days). No local tumour was observed (Neukomm & de Trey, 1961).

In a study reported in an abstract, female Marsh mice, three months old, received single subcutaneous injections of 20 mg USP talc and were observed for 18-21 months. No tumour developed at the injection site in 26 treated animals or in 24 saline-injected controls (Bischoff & Bryson, 1976).

(e) *Intraperitoneal administration*

Mouse: In a study reported in an abstract, female Marsh mice, three months old, received single intraperitoneal injections of 20 mg USP talc and were observed for 18-21 months. Intraperitoneal lymphoid tumours occurred in 5/22 treated animals and in 6/28 saline-treated controls (Bischoff & Bryson, 1976).

Forty Swiss albino mice [sex unspecified], six weeks of age, received single intraperitoneal injections of 20 mg ground commercial talc [unspecified] in saline. Before six months, 16 animals had died. In the 24 survivors allowed to live out their normal lifespan [unspecified], three peritoneal mesotheliomas were observed, compared with 3/46 in saline-treated controls (Özsmi *et al.*, 1985). [The Working Group noted the inadequate reporting of the study.]

Rat: A group of 40 female Wistar rats, eight to 12 weeks of age, received four intraperitoneal injections of 25 mg granular talc in 2 ml saline at weekly intervals. A group of 80 female rats injected with saline served as controls. The rats were observed until spontaneous death or sacrifice (average survival time after injection, 602 days). A mesothelioma was observed in 1/36 talc-exposed rats after 587 days compared with none in 72 controls (Pott *et al.*, 1974, 1976a,b).

In a study reported in an abstract, three-month-old female Evans rats received single intraperitoneal injections of 100 mg USP talc and were observed for 18-21 months. Of the treated rats, 3/27 developed tumours (one lymphosarcoma, one reticulum-cell sarcoma in the peritoneal cavity, one cystadenoma of the liver), compared with none in 26 saline-treated controls (Bischoff & Bryson, 1976).

(f) *Intrapleural and intrathoracic administration*

Mouse: In a study reported in an abstract, male Marsh mice, three months old, received single intrathoracic injections of 10 mg USP talc. After 18-21 months, 5/47 treated mice had tumours (two adenocarcinomas and three lymphoid tumours of the lung), compared with none of 48 saline-injected controls (Bischoff & Bryson, 1976).

Rat: In a study reported in an abstract, female Evans rats, three months old, received single intrathoracic injections of 50 mg USP talc. After 18-21 months, intrathoracic reticulum-cell sarcomas or lymphomas were observed in 7/30 talc-treated rats, in 8/32 saline-treated animals and in 7/28 untreated controls (Bischoff & Bryson, 1976).

A group of 24 male and 24 female Wistar-derived rats, eight to 14 weeks old, received single intrapleural injections of 20 mg Italian talc (grade 00000; ready milled; mean particle size, 25 μ m; containing 92% talc, 3% chlorite, 1% carbonate minerals and 0.5-1% quartz). The mean survival time of the treated rats (655 days) was similar to that of 24 male and 24 female controls (691 days) injected with saline. No mesothelioma was detected in either group; one small pulmonary adenoma was found in one rat that died 25 months after injection (Wagner *et al.*, 1977).

Groups of 30-50 female Osborne-Mendel rats, 12-20 weeks old, received single intrapleural implantations of 40 mg of one of seven grades of refined commercial talc from separate sources in hardened gelatin. The rats were followed for two years, at which time survivors were killed. The incidences of pleural sarcomas were: talc 1, 1/26; talc 2, 1/30; talc 3, 1/29; talc 4, 1/29; talc 5, 0/30; talc 6, 0/30; talc 7, 0/29; compared with 3/491 in untreated controls, 17/615 in controls receiving implants of 'nonfibrous' materials described by the authors as 'noncarcinogenic' and 14/29 in rats receiving UICC crocidolite asbestos (Stanton *et al.*, 1981).

3.2 Other relevant biological data

(a) *Experimental systems*

Toxic effects

A review of the literature prior to 1978 on the biological effects of talc is available (Lord, 1978).

The Working Group noted that in most of the studies of 'talc' described below, no or limited characterization of the mineralogy of the sample employed was given, and, in particular, there was a lack of information on fibre content or particle size.

(i) *Lethality*

The LD₅₀ of talc has not been established unequivocally.

Significant mortality was observed in guinea-pigs after two or three intravenous injections of 25 mg talc in saline (Dogra *et al.*, 1977). In contrast, there was no treatment-related death in rabbits injected intravenously daily for two weeks with 100 mg talc in saline (Puro *et al.*, 1966), in rabbits receiving twice-weekly intravenous injections of 50 mg talc for ten weeks or in rats receiving twice-weekly intravenous injections of talc over a nine-week period (total dose, 100 mg) (Schepers & Durkan, 1955b). Three of 11 rats died within one day following injection of 1400 mg/kg bw talc into the lower pole of the spleen (Eger & Da Canal, 1964).

In most of the studies described below, no acute mortality was observed in several species of animals following administration of high doses of talc by ingestion, inhalation or intratracheal, intrapleural, intraperitoneal or subcutaneous injection.

In rats fed 100 mg talc per day for 101 days, no significant depression of mean lifespan was observed (Wagner *et al.*, 1977).

Several studies of exposure to talc *via* inhalation have been reported; but, until recently (see Hanson *et al.*, 1985), the primary technical problem associated with inhalation experiments has been a lack of methods to determine accurately the amount of talc inhaled by exposed animals. The acute mortality observed in rats exposed to a 'very dense' cloud of talc (particle size, $<5 \mu\text{m}$) for 3 h per day for up to 12 days may have been due to suffocation (Policard, 1940). None of a group of rats exposed to 30-383 mg/m^3 'technical'- or 'pharmaceutical'-grade talc for 6 h per day on six days per week for up to nine months died as a specific consequence of exposure (Bethge-Iwańska, 1971). No effect was observed on the survival of hamsters exposed by inhalation to 8 mg/m^3 respirable 'baby talc' for up to 150 min per day on five days per week for 300 days (Wehner *et al.*, 1977a, 1979).

A 79% mortality rate was reported in rats receiving a single intratracheal injection of 50 mg/ml talc in water. Subsequently, it was found that rats could tolerate the dose if they were given two injections of 25 $\text{mg}/0.5 \text{ ml}$ at weekly intervals (Lüchtrath & Schmidt, 1959). A 40% mortality was observed in rats injected intratracheally with 25 mg tremolitic talc/ ml water (Gross *et al.*, 1970). Low mortality (2/14) was reported in chinchillas given five intratracheal injections of 40 mg talc in saline (both deaths occurred after the first injection) (Trautwein & Helmboldt, 1967).

No significant mortality was observed following intrapleural injection of 20 mg talc in saline into rats (Wagner *et al.*, 1977). Increased mortality was reported in mice six months after intraperitoneal injection of 20 mg 'commercial' talc in saline (Özesmi *et al.*, 1985), but no increased mortality was observed in rats injected intraperitoneally with 100 mg talc in saline (Pott *et al.*, 1976a). No acute toxicity was observed after a single injection of 10 mg into the bursa of rats (Hamilton *et al.*, 1984) or after suprascapular subcutaneous injection of 600 mg into mice (Carson & Kaltenbach, 1973). Transient convulsions were observed in rabbits following cisternal injection of 1 ml of a 1:9 or 1:4 suspension of talc in saline (Oppenheimer & Riester, 1953).

(ii) *Chronic toxicity*

Mild to marked arterial endothelial cell proliferation with cellular encroachment into the lumen and the occurrence of occasional foreign-body giant cells within the endothelial masses were observed after daily intravenous injections of 100 mg talc for two weeks to rabbits (Puro *et al.*, 1966). After three intravenous doses of 25 mg talc in saline to guinea-pigs, mild proliferation of the endothelial cells and moderate thickening of the intra-alveolar septa of the lungs were observed 150 days after injection (Dogra *et al.*, 1977). In contrast, no effect on the rat lung was observed after intravenous injection of talc (Schepers & Durkan, 1955b). Talc granulomas were observed in the region of Glisson's capsule following intrasplenic injection of 1400 mg/kg talc to rats (Eger & Da Canalis, 1964). After cisternal injection of talc to rabbits, no permanent neurological disorder was

seen. Microscopic examination revealed a phagocytic, histiocytic response, with some fibroblastic proliferation and dense adhesions between the membranes (Oppenheimer & Riester, 1953).

No chronic pathological effect was associated with oral administration of talc to rats (Wagner *et al.*, 1977). Intratracheal injections of talc (total dose, 150 mg) to guinea-pigs induced perivascular and peribronchiolar focal accumulations of histiocytes, fibrocytes, plasma cells and eosinophils within one month; by eight months, some fibrosis, with fibrocellular sclerosis of the pleural surface, was observed. After two years, the dominant effects were bronchiolectasia, bronchiolitis and marked fibrosis (Schepers & Durkan, 1955b).

No evidence of lung fibrosis or lymph node abnormality was observed in rats given a single intratracheal injection of 50 mg 'pure' talc in water; however, rats that received the same dose of 'calcined' (1000-1100°C) talc developed lung and lymph node fibrosis after 13 months (Lüchtrath & Schmidt, 1959). Proliferative inflammation of the smaller bronchi and bronchioles was observed in rats four days after intratracheal injection of 25 mg talc (containing tremolite; fibres, 0.1-0.2 μm in diameter) in water; within a few months, collagenous tissue had been formed (Gross *et al.*, 1970).

Chinchillas receiving a single or several intratracheal injections of 40 mg 'purified' talc in saline exhibited chronic pulmonary irritation and proliferative pneumonia, with giant-cell granulomas and adjacent metaplasia of the alveolar epithelium. The hyperplastic cells subsequently transformed into cuboid cells that formed a continuous lining of the affected alveoli and finally acquired an adenomatous appearance (Trautwein & Helmboldt, 1967).

Exposure by inhalation to a 'heavy dosing' of talc was badly tolerated by rats, causing severe dyspnoea. However, no histological change was observed within 20 days, and talc particles were trapped by alveolar macrophages (Policard, 1940). Rats exposed to dust clouds of 30-383 mg/m^3 'industrial'- or 'pharmaceutical'-grade talc for nine months developed chronic inflammatory changes, including thickening of the pulmonary arteries walls and, eventually, emphysema (Bethge-Iwańska, 1971).

In rats exposed by inhalation to 10.8 mg/m^3 Italian talc (grade 00000; ready milled; mean particle size, 25 μm) for three months, minimal fibrosis was observed, the degree of which did not change during the post-exposure period. Animals exposed for one year had minimal to slight fibrosis, the degree of which had increased to moderate within one year after cessation of exposure (Wagner *et al.*, 1977). In contrast, Syrian golden hamsters exposed to 8 mg/m^3 talc aerosols for up to 150 min per day on five days per week for 30 days showed no histopathological change in the lungs, heart, liver, renal tissues, stomach or uterus (Wehner *et al.*, 1977a, 1979; Wehner, 1980).

Injection of 10 mg talc (containing some asbestos fibres) into the pleural cavity of mice has been reported to produce granulomas, some of which were firmly attached to the surface of the lungs or other chest contents and, occasionally, to the lung lobes (Davis, 1972). Two years after injection of 20 mg Italian talc (see above) into the right pleural cavity of rats, granulomas at the injection site were common, and one small pulmonary adenoma was observed, but no other relevant pathology was observed in the lungs (Wagner *et al.*, 1977).

Guinea-pigs received single intraperitoneal injections of 200 mg of one of seven 'industrial'-grade talcs (up to 52% talc, up to 82% tremolite, traces of quartz). Nodules consisting of macrophages and giant cells were first observed at ten days on the ventral parietal surface and over a 15-month period became smaller. Fibroblastic proliferation was pronounced in the early phases (Schulz & Williams, 1942).

Six months after intraperitoneal injection of approximately 400 mg of a talcum powder used on surgical gloves, laparotomized albino rats exhibited typical granulomas with numerous foreign-body giant cells (Blümel *et al.*, 1962). These findings were confirmed in rats implanted with suture material dusted with talc or talc pellets, which resulted in a chronic inflammatory process with persistent granuloma formation (Sheikh *et al.*, 1984).

(iii) *Toxicity in vitro*

The concentration of talc (99% pure) required to cause 50% haemolysis of red-blood cells was 65 mg/ml, which is more than 50 fold that of chrysotile (Woodworth *et al.*, 1982).

Mouse peritoneal macrophages were exposed to seven different specimens of talc (only one of which contained amphibole fibres); all seven were found to be 'modestly' cytotoxic, as determined by the release of lactate dehydrogenase and β -glucuronidase, to a degree ten-fold less than quartz. No statistical difference was reported for the effects of the different talc samples (Davies *et al.*, 1983). The phagocytosis of talc by rabbit lung fibroblasts has been reported (Henderson *et al.*, 1975a).

A concentration of 0.1 mg/ml talc (99% pure) caused 35% release of ^{51}Cr from Syrian hamster tracheal epithelial cells labelled with sodium chromate; the concentration is two-fold that required for chrysotile (Woodworth *et al.*, 1982).

A concentration of $>50 \mu\text{g/ml}$ Italian talc caused a 50% reduction in the colony-forming efficiency of Chinese hamster V79-4 lung cells (Chamberlain & Brown, 1978).

Effects on reproduction and prenatal toxicity

Talc was found to produce nonspecific abnormalities in chicken eggs, at an incidence similar to that induced by thalidomide and sulphadimethoxine (Carter, 1965; Yang, 1977).

No teratological effect was observed in hamsters, rats, mice or rabbits following oral administration of talc. The doses used were 1600 mg/kg bw to rats and mice on days 6-15 of gestation; 1200 mg/kg bw per day to hamsters on days 6-10 of gestation; and 900 mg/kg bw to rabbits on days 6-18 of gestation (Food and Drug Research Laboratories, 1973).

Deposition, retention and clearance

The deposition, translocation and clearance of talc in hamsters was followed by giving them a single nose-only inhalation exposure for 2 h to 40-75 mg/m³ neutron-activated talc (median diameter based on radioactivity measurements, 6.4-6.9 μm). High-grade cosmetic talc was used, consisting of 95% (w/w) platy talc mineral. Alveolar deposition was approximately 20-80 μg , representing 6-8% of the inhaled amount. The biological half-life of the talc deposited in the alveoli was seven to ten days, and alveolar clearance was reported to be essentially complete four months after exposure. [The Working Group noted that the unusually short clearance time may relate to limitations in the sensitivity of the detection

methods and the large size of the particles used.] No translocation of talc to liver, kidneys, ovaries or other parts of the body was found (Wehner *et al.*, 1977a,b).

In rats exposed to aerosols (mean respirable dust, 10.8 mg/m³) of Italian talc (see above), the mean amounts of talc retained in the lung were 2.5, 4.7 and 12.2 mg per rat following exposures for three, six and 12 months, respectively. These levels were roughly proportional to the cumulative exposures (Wagner *et al.*, 1977). In rats exposed for 6 h per day on five days per week for four weeks to 2.3, 4.3 and 17 mg/m³ respirable talc, the amounts retained in the lung at the end of exposure were 77, 187 and 806 µg talc per g lung, respectively (Hanson *et al.*, 1985).

Talc, like other foreign particles, has been found to depress the clearance of 3,4-benzo[*a*]pyrene from the lungs of hamsters (Pelfrene, 1976).

Guinea-pigs were given a single intraperitoneal injection of 200 mg of one of seven commercial talc samples (containing 3-52% talc, the rest being serpentines, carbonate, quartz and tremolite; 82% tremolite in one sample) and were examined at intervals up to 15 months. Because of differences in solubility, there was relative enrichment of the sample with talc. Talc particles were found mainly on the ventral parietal surface of the peritoneum within macrophages and giant cells (Schulz & Williams, 1942).

In studies in rats, mice, guinea-pigs and hamsters using radioactive tracer techniques, no intestinal absorption or translocation of ingested talc to the liver and kidneys was detected (Wehner *et al.*, 1977c; Phillips *et al.*, 1978). No translocation of talc into the ovaries was detected after single or multiple intravaginal applications to rabbits (Phillips *et al.*, 1978).

Mutagenicity and other short-term tests

Talc was not mutagenic to *Salmonella typhimurium* TA1530 or *his* G46 or to *Saccharomyces cerevisiae* D3 *in vitro* [full details not given] or in host-mediated assays in mice (30-5000 mg/kg bw) (Litton Bionetics, 1974).

Chromosomal aberrations were not induced in human WI38 cells treated with talc at 2-200 µg/ml, and neither chromosomal aberrations nor dominant lethal mutations were induced in rats following oral administration of 30-5000 mg/kg bw talc (Litton Bionetics, 1974).

Single intraperitoneal injections of 20 mg talc plus 2 mg particulate prednisolone acetate in saline into mice induced significant numbers of multinucleated giant cells within 48 h. Neither compound alone induced this response. The multinucleate cells arose by cell fusion, and the resultant polykarions exhibited severe structural chromosomal abnormalities (bridges, acentrics and dispersed chromosomes). Prednisone in combination with talc also elicited the formation of multinucleated giant cells. Polykarions were not observed when talc was injected in combination with cortexone acetate, cortisone or testosterone isobutyrate (Dreher *et al.*, 1978).

(b) Humans

Toxic effects

The toxic effects of talc are dependent on the route, dose and properties of the talc

involved. In addition, talc commonly contains other minerals (see section 1.3), including in some instances several forms of asbestos and silica.

Talc pneumoconiosis is somewhat more prevalent and severe among people exposed to talc containing asbestiform minerals than among those exposed to talc without such impurities (Schepers & Durkan, 1955a; Kleinfeld *et al.*, 1963). The form of the pneumoconiosis varies widely, from an asymptomatic simple type (Buus-Hansen *et al.*, 1950; Vallyathan & Craighead, 1981) to disabling conglomerate pneumoconiosis (Jaques & Benirschke, 1952; Hunt, 1956; Graham & Gaensler, 1965; Fristedt *et al.*, 1968; Miller *et al.*, 1971). Mixed-dust pneumoconiosis is frequently seen, including silicosis, asbestosis and occasionally other forms (Porro *et al.*, 1942; Schepers & Durkan, 1955a; Kleinfeld *et al.*, 1963; Mark *et al.*, 1979).

Several early reports describe 'talcum powder granuloma' arising from the use of talc on surgical gloves (Antopol, 1933; Fienberg, 1937; German, 1943; Eiseman *et al.*, 1947; Diffenbaugh, 1953; Henderson *et al.*, 1975b). Subsequent cases have been reported which document a variety of surgical complications, including adhesions, pseudotumours and sinus tracts attributable to talc exposure (Lichtman *et al.*, 1946; Pruvost, 1946; Eiseman *et al.*, 1947; Saxén & Tuovinen, 1947; Enderlin *et al.*, 1959). Both skin granulomas and talc pneumoconiosis have been reported after liberal use of talc on the body (Tye *et al.*, 1966; Nam & Gracey, 1972; Wells *et al.*, 1979; Tukiainen *et al.*, 1984).

Respiratory distress syndrome, which can be fatal, has been described in children following massive accidental inhalation of talcum powder (Cless & Anger, 1954; Molnar *et al.*, 1962; Gouvêa *et al.*, 1966; Hughes & Kalmer, 1966; Lund & Feldt-Rasmussen, 1969; Niemann *et al.*, 1971; Gould & Barnardo, 1972). Acute bronchitis and bronchiolitis were found in a 22-month-old boy who died following accidental inhalation of talc (Molnar *et al.*, 1962).

A variety of pathological effects arise from intravenous use of talc containing drugs by addicts. These include micronuclear pulmonary opacities (Krainer *et al.*, 1962; Hopkins & Taylor, 1970; Szwed, 1970; Arnett *et al.*, 1976; Smith *et al.*, 1978; Waller *et al.*, 1980; Tao *et al.*, 1984), angiothrombotic pulmonary hypertension (Wendt *et al.*, 1964; Bainborough & Jericho, 1970; Zientara & Moore, 1970; Arnett *et al.*, 1976; Paré *et al.*, 1979; Waller *et al.*, 1980) and conglomerate pulmonary lesions (Sieniewicz & Nidecker, 1980; Crouch & Churg, 1983). Reduced pulmonary function has also been observed (Paré *et al.*, 1979). In addition, retinopathy, cerebral microembolization and granulomas of the liver, lymph nodes and kidneys have been reported (Lee & Sapira, 1973; Min *et al.*, 1974; Paré *et al.*, 1979; Carman, 1985).

Two studies by the US Public Health Service (Dreessen, 1933; Dreessen & DallaValle, 1935) of talc containing tremolite showed a high prevalence of pneumoconiosis in workers in talc mines and mills, which appeared to be related to dust concentration and duration of exposure. A variety of pneumoconiotic effects was seen, which did not appear to be related to differences in tremolite content. A series of cross-sectional studies reported from the New York State Department of Labor (Kleinfeld *et al.*, 1955; Messite *et al.*, 1959; Kleinfeld *et al.*, 1963, 1964a,b, 1973) have documented a high prevalence of talc pneumoconiosis in talc miners and millers, especially among tremolitic talc workers. The cases were associated

with pleural plaques, restrictive or obstructive breathing disorders and decreased vital capacity. The prevalence of disease was lower among those with lower cumulative dust exposure and among those processing granular rather than fibrous talc. A large, well-controlled, industry-wide study of miners and millers in four talc deposits in the USA (Gamble *et al.*, 1979a,b; Dement *et al.*, 1980; Gamble *et al.*, 1982) revealed associations between talc containing tremolite and anthophyllite and increased prevalence of bilateral pleural thickening, which was also associated with significant reductions in lung function.

A series of cross-sectional studies describing talc pneumoconiosis in workers in talc mining, milling and manufacture in Italy (Rubino *et al.*, 1963; Tronzano *et al.*, 1965) found that the prevalence was related to extent and duration of exposure and that talcs contaminated with tremolite, serpentine and quartz were associated with significant pneumoconiosis. Similarly, in studies in Egypt (El Ghawabi *et al.*, 1970; Emara *et al.*, 1984), a high prevalence of pneumoconiosis was associated with heavy exposure to talc during milling and in the cosmetics industry; obstructive and restrictive pulmonary impairment were seen among persons with pneumoconiosis.

One reasonably large, representative, well-controlled study of exposure in the rubber industry to Vermont talc, reported to have a low content of silica and fibres, showed significantly increased respiratory symptoms and impaired ventilatory function but no radiographic abnormality (Fine *et al.*, 1976).

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Deposition, retention and clearance

Talc particles have been found at autopsy in the lungs of cases of 'talc pneumoconiosis' (Schepers & Durkan, 1955a; Seeler *et al.*, 1959; Kleinfeld *et al.*, 1963; Berner *et al.*, 1981; Vallyathan & Craighead, 1981). Talc, in the form of platy or elongated particles, has been found at autopsy in the lungs of urban residents, farmers, asbestos miners and drug addicts (Seeler *et al.*, 1959; Langer *et al.*, 1971; Pooley, 1976; Abraham & Brambilla, 1979; Gylseth *et al.*, 1984). It has been reported to be concentrated in lung scar tissue (Yao *et al.*, 1984).

Churg and Wiggs (1985) analysed by transmission electron microscopy and energy dispersive X-ray spectroscopy the total fibrous and nonfibrous mineral content of the lungs of a series of 14 male smokers with lung cancer but with no history of occupational dust exposure, and of a series of 14 control men matched by age, smoking history and general occupational class. The average concentrations of mineral fibres and nonfibrous particles were 3.8 and 2.0 times higher in the group with cancer. Kaolinite, talc, mica, feldspars and crystalline silica comprised the majority of fibrous and nonfibrous particles in both groups.

Talc particles were found in stomach tumours from Japanese men (Henderson *et al.*, 1975c), possibly due to ingestion of talc-treated rice (Merliss, 1971a,b). Talc particles, but apparently no other insoluble particle, were found in the subserosal stroma of hernia sacs, possibly due to ingestion of medications in which talc is present as a filler (Pratt *et al.*, 1985).

Talc is used as a filler in some materials that drug addicts inject, resulting in wide dissemination of talc particles to the lung (Groth *et al.*, 1972; Lamb & Roberts, 1972; Farber *et al.*, 1981; Crouch & Churg, 1983), spleen, kidney, liver, brain, heart, adrenal and thyroid (Groth *et al.*, 1972) and even the retina (AtLee, 1972). In lung, most of the talc particles are seen within the vessels of the alveolar walls, and are almost invariably associated with marked foreign body granulomas (Crouch & Churg, 1983). The talc particles found in the lung are larger after intravenous injection than after inhalation (Abraham & Brambilla, 1979).

Mutagenicity and chromosomal effects

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity to humans

(a) Case reports and case series

Individual case reports of cancer include a lung adenocarcinoma two years following talc pleurodesis (Jackson & Bennett, 1969) and a pleural mesothelioma following occupational exposure to talc (Chahinian *et al.*, 1982; Barz & Beck, 1983; Barnes & Rogers, 1984). [The Working Group noted that either these cases were associated with evidence of asbestos exposure or insufficient environmental data were available to determine whether asbestos exposure had occurred (Chahinian *et al.*, 1982).]

Four cases of mesothelioma reported to the tumour registry of the Cancer Control Bureau, New York Department of Health, USA, were associated with exposure to talc mining. Talc mines in St Lawrence County, New York, contain high levels of fibrous tremolite, the suggested etiological agent (Vianna *et al.*, 1981).

A survey of the long-term effects of talc and kaolin pleurodesis was reported by the Research Committee of the British Thoracic Association and the Medical Research Council Pneumoconiosis Unit (1979). No increase in the number of lung cancer deaths was observed, and no case of mesothelioma was reported. [The Working Group noted that there are several methodological limitations, including the fact that the duration of follow-up was less than 15 years, no data were available on smoking, and no specific information was given on the type or source of talc used.]

(b) Epidemiological studies

Kleinfeld *et al.* (1967, 1974) reported two studies on New York talc miners and millers, the results of which are substantially the same; the more complete 1974 results are reported here. Men employed in 1940, who had accumulated 15 or more years of exposure to commercial talc dust as well as those who achieved a minimum of 15 years of such exposure between 1940 and 1969, were included in this study. The cohort totalled 260 workers and was believed to represent the total work force meeting the exposure criteria. Proportionate mortality was calculated utilizing US white male mortality for the year 1955, the median year of the 108 deaths observed. Environmental exposure was reported to be predominantly to talc containing tremolite and anthophyllite (asbestiform and nonasbestiform habits),

carbonate dusts and a small amount of free silica. Further dust counts were provided for the years 1966-1969: mines had median counts ranging from 9-19 mppcf, and mills, 20-24 mppcf; dust counts and fibre counts reported for the year 1972 ranged from 3-7 mppcf and 2-3 fibres/cm³ in mines and 7-28 mppcf and 24-62 fibres/cm³ in mills. Mortality from lung and pleural cancer showed a three-fold overall increase: observed, 12%; expected, 3.7%. No significant excess was found for gastrointestinal cancers. One peritoneal mesothelioma was noted. [The Working Group noted that, as for the previously reported proportionate mortality study (Kleinfeld *et al.*, 1967), no data were available on smoking or on cumulative dose in individual workers; nor were further data given about the distribution of workers among the several mines and mills from which these records were extracted.]

A cohort mortality study was conducted of 398 white men initially employed between 1 January 1947 and 31 December 1959 in mining and milling talc in the Gouverneur Talc District of Upper New York State (St Lawrence County) (Brown *et al.*, 1979; Dement *et al.*, 1980). In addition to talc, the product contained tremolite, anthophyllite and serpentine minerals, some of which were asbestiform. [Further details of the exposure are reported in section 2.2(b).] Vital status was ascertained as of 1975. Fifty percent of the workers had been employed less than one year and 27% for ten years or more. Statistically significant excesses in mortality were observed for all malignant neoplasms (19 observed, 10.6 expected; standardized mortality ratio [SMR], 180), for neoplasms of the respiratory system (10/3.5; SMR, 290), for bronchogenic cancer (9/3.3; SMR, 270) and for all nonmalignant respiratory disease (8/2.9; SMR, 277). Evidence of an exposure-response relationship was observed by latency for bronchogenic cancer. The authors concluded that tremolite and anthophyllite are the prime suspected etiological factors associated with the observed increase in bronchogenic cancer and nonmalignant respiratory disease in this cohort. No data on smoking were available. A possible confounding factor in this study was previous exposures at other mines in the area; however, exposures to amphibole fibre in all these regional talc operations were reported to be substantially the same.

Stille and Tabershaw (1982) conducted a cohort mortality study on the same mine and mill studied by Brown *et al.* (1979). The composition of their cohorts was somewhat different, the current study including 655 employees who had ever worked for the company between 1 January 1948 and 31 December 1977, after exclusion of 35 women office workers and 53 workers for whom birth dates or other significant data were not available. Cause-specific mortality rates were based on 113 deaths as of December 1978. The SMR for all sites of cancer was 122 (25 observed/20.5 expected); 11 cases were respiratory cancers, and ten of those were lung cancer, with SMRs of 163 and 157, respectively. The cohort was then divided according to whether an individual had been employed elsewhere before coming to work at the particular mine and mill under investigation. Those few who had worked only at the company in question were found to have very low mortality from lung cancer (two observed, 2.6 expected). [The Working Group noted a number of methodological problems, including selection bias, lack of statistical testing, small numbers of person-years of exposure, and no analysis with respect to exposure.]

Rubino *et al.* (1976) studied 1514 miners and 478 millers employed for at least one year between 1921 and 1950 in talc mines and mills in the Germanasca and Chisone valleys

(Piedmont) in Italy. The talc in those mines is described as quite pure, with only some tremolite microinclusions; no other fibrous mineral was reportedly found. [Further details of the exposure are reported in section 2.2(b).] Significant increases in specific cause of death among miners were found for silicosis (62 observed/30.9 expected) and for silico-tuberculosis (18/9.1). Significant deficits in cause-specific mortality were reported for malignant neoplasms (100/129.5), malignant neoplasms of the lung, bronchus and trachea (9/19.7) and malignant neoplasms at other sites (23/39.9). Two cases of pleural mesothelioma and a high occurrence of silicosis and silico-tuberculosis were found in the comparison group. [The Working Group noted that the method used to derive the number of expected deaths is not adequately described. It was considered that the lack of comparability between the worker and comparison groups could be the main explanation for the mortality increases and deficits observed in this study.]

Selevan *et al.* (1979) carried out a study of talc exposures in five companies (two of which ceased operations in 1952 and 1960) in three regions in Vermont, USA. Analysis of airborne dust samples and talc bulk samples revealed no asbestos, either by X-ray diffraction or analytical electron microscopy. Levels of respirable free silica were below 0.25% in nearly all ore and product samples, and free silica was only occasionally detectable in air samples. Insufficient information was available to estimate cumulative exposures, but the authors stated that past exposure levels for miners and millers far exceeded the present standard for nonfibrous talc of 20 mppcf. They considered it probable that dust exposures for millers were higher than those for miners. In one mine, which had closed by the time of the study, 'cobblestones' of highly tremolitic serpentine rock were present but were avoided or discarded as far as possible prior to milling. The cohort consisted of all white male talc workers who had been radiographed as part of annual voluntary surveys of the Vermont Health Department, who were employed in the Vermont talc industry between 1 January 1940 and 31 December 1969, and who had worked in the industry for at least one year. [Because of the voluntary nature of the survey, the cohort may not have been representative (Davis *et al.*, 1983).] There were 90 deaths among the 392 members of this cohort; vital status was not established for four. For nonmalignant respiratory disease and respiratory cancer, Vermont rates were used for comparison, because they are higher than national rates; for other causes of death, US rates were used. [The Working Group noted this unconventional analytical approach.] While some increase was noted for malignant neoplasms, and specifically for respiratory neoplasms (6 observed/3.69 expected), these were not found to be significant. [The Working Group noted that the results were not analysed by latency.] The excess of respiratory cancer occurred only among miners (5/1.15; $p < 0.05$), and the significant excess for nonmalignant respiratory disease occurred only among millers (7/1.72; $p < 0.01$). Most of those dying with nonmalignant respiratory disease had radiographic evidence of pneumoconiosis (rounded opacities). Miners were also exposed to radon daughters at mean levels ranging up to 0.12 working levels, with single peaks of 1.0 working level. [The Working Group noted that no data on smoking were available.]

In a short communication, Léophonte *et al.* (1983) reported on the mortality of talc workers in Luzenac, France. The talc in this region is said to contain no asbestos and levels of quartz varying from 0.5 to 3%. The cohort comprised those who left employment between

1 January 1945 and 31 December 1981 having worked for at least one year. Of 470 workers available for study, 256 were living, 209 had died and five were lost to follow-up; 192/204 with known occupational exposure had worked only at Luzenac. When compared with the regional population, the median age of death was not found to be influenced by dust exposure. There was no significant excess in cancer mortality in general, and, specifically, mortality from respiratory and digestive cancers was not increased. A significant increase in mortality was found for nonmalignant respiratory disease, especially for pneumoconiosis and obstructive lung disease. [The Working Group noted the unconventional definition of the cohort, that no data on smoking habits were available, and that causes of death were obtained for cases from local doctors, hospitals or families but for controls from regional or national records.]

Katsnelson and Mokronosova (1979) reported a study of mortality among workers in a talc mining and processing plant in the USSR. Very high mortality ratios were found. [The Working Group noted that the deaths observed among exposed workers included current and past workers but that the denominator comprised only currently employed persons.]

It has been suggested on the basis of ecological studies that the practice of coating rice with talc, which may be contaminated with asbestos, may play a causal role in the relatively high rate of stomach cancer in Japan (Merliss, 1971a,b; Blejer & Arlon, 1973; Matsudo *et al.*, 1974); however, this hypothesis has not been supported by case-control studies.

Cramer *et al.* (1982) reported a case-control study of ovarian cancer and talc exposure in the Boston, Massachusetts, USA, area between November 1978 and September 1981. Two-hundred-and-fifteen women with pathologically-confirmed epithelial ovarian cancers were identified and matched randomly by residence, race and age. Ninety-two (42.8%) cases regularly used talc either as a dusting powder on the perineum or on sanitary napkins compared with 61 (28.4%) controls. Adjusted for parity and menopausal status, this difference yields a relative risk of 1.9 ($p < 0.003$). Women who had regularly engaged in both practices had an adjusted relative risk of 3.3 ($p < 0.001$) compared to women with neither exposure. [The Working Group noted that while this study suggests an association between talc use and ovarian cancer, information was not available regarding the asbestos content of the talcs, levels of exposure or whether the interviews were conducted by people who were unaware of the case referent status of the person being interviewed.]

4. Summary of Data Reported and Evaluation

4.1 Exposure data

Talc occurs in various geological settings around the world but is usually formed by alteration of ultramafic rocks or dolomites. Talc deposits may contain various other minerals, including carbonates, free silica and serpentines (including chrysotile) and amphibole minerals (asbestiform and nonasbestiform). Occupational exposures occur during mining, milling, processing and in a wide variety of secondary industries (e.g.,

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ceramics, paper, rubber and paint production). Exposure of the general population occurs through use of products such as cosmetics.

4.2 Experimental data

Talc of different grades was tested for carcinogenicity in mice by subcutaneous, intraperitoneal and intrathoracic injection, in rats by oral administration, inhalation exposure and intraperitoneal, intrathoracic and intrapleural injection, and in hamsters by inhalation exposure and intratracheal instillation. The majority of these studies were inadequate. Tumour incidence was not increased following either the administration of single doses of various talcs to rats by intrapleural administration or administration of talc by four intraperitoneal injections. A single subcutaneous injection of talc in mice did not produce local tumours. No tumour was produced by administration of talc in the diet of rats. In most of the above studies, characterization of the talc was insufficient to determine whether it contained asbestiform fibres.

No teratogenic effect was observed in rats, mice, hamsters or rabbits following oral administration of talc.

Talc was not mutagenic to *Salmonella typhimurium* or *Saccharomyces cerevisiae* in host-mediated assays. It did not induce chromosomal aberrations in cultured human cells or in rats *in vivo* or dominant lethal mutations in rats.

Overall assessment of data from short-term tests: Talc^a

	Genetic activity			Cell transformation
	DNA damage	Mutation	Chromosomal effects	
Prokaryotes		—		
Fungi/ Green plants		—		
Insects				
Mammalian cells (<i>in vitro</i>)			—	
Mammals (<i>in vivo</i>)			—	
Humans (<i>in vivo</i>)				
Degree of evidence in short-term tests for genetic activity: Inadequate				Cell transformation: No data

^aThe groups into which the table is divided and the symbol '—' are defined on pp. 19-20 of the Preamble; the degrees of evidence are defined on pp. 20-21.

4.3 Human data

Case reports have suggested an association between exposure to talc containing asbestiform fibres and mesothelioma.

Proportionate mortality studies of miners and millers of talc containing asbestiform tremolite and anthophyllite showed an excess of lung cancer and one case of mesothelioma. A cohort study of workers in one company revealed significant excess mortality from lung cancer and from nonmalignant respiratory disease. Mortality from lung cancer increased with latency.

In several mortality studies, cancer risk was assessed among miners and millers of talc that was reported to contain no more than trace amounts of asbestiform minerals. A cohort mortality study of talc miners and millers showed an excess of lung cancer in underground miners but not in millers; a contributory etiological role of radon daughters to the lung cancer risk in miners could not be excluded. Three other studies suffered from methodological limitations and could not be interpreted.

A case-control study suggested an approximate doubling of the risk for ovarian cancer among women after perineal use of talc.

4.4 Evaluation¹

There is *inadequate evidence* for the carcinogenicity of talc to experimental animals.

There is *inadequate evidence* for the carcinogenicity to humans of talc not containing asbestiform fibres, while there is *sufficient evidence* for the carcinogenicity to humans of talc containing asbestiform fibres.

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¹For definition of the italicized terms, see Preamble, pp. 18 and 22.

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Exhibit 101

to be informed promptly and effectively of important new knowledge regarding nutritional and health benefits of food. Third, these amendments to this health claim will ensure that scientifically sound nutritional and health information regarding the benefits of fruit and vegetable intake and reduction of CHD risk can be provided to consumers as soon as possible. The past few editions of the DGA have been moving away from a focus on total fat and have instead communicated to consumers the need to focus on type of fat consumed instead of total amount of fat. Recent editions of the DGA have also encouraged increased intake of fruits and vegetables for a healthful diet. Prompt issuance of an interim final rule that reflects the current recommendations is necessary for consumers to be able to have the most current information on nutrition and diet. Consumers will be better able to construct healthful diets if they have prompt access to information that is consistent with the current recommendations on fat content and on consumption of fruits and vegetables. Therefore, we are using the authority in section 403(r)(7)(A) of the FD&C Act to issue an interim final rule amending the general requirements for the health claim for dietary saturated fat and cholesterol and risk of CHD and to make the interim final rule effective immediately.

This regulation is effective upon publication in the **Federal Register**. We invite public comment on this interim final rule. We will consider modifications to this interim final rule based on comments made during the comment period. We will address comments and confirm or amend the interim final rule in a final rule.

X. References

The following references are on display in the Division of Dockets Management (see **ADDRESSES**) and are available for viewing by interested persons between 9 a.m. and 4 p.m., Monday through Friday; they are also available electronically at <http://www.regulations.gov>. FDA has verified the Web site addresses, as of the date this document publishes in the **Federal Register**, but Web sites are subject to change over time.

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8. Institute of Medicine (IOM) of the National Academies. "Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)." Chapter 8, "Dietary Fats: Total Fat and Fatty Acids," 2002.

9. FDA/CFSAN, Food Labeling: Health Claims; Dietary Saturated Fat and Cholesterol and Risk of Coronary Heart Disease, Regulatory Impact Analysis, FDA–2013–P–0047.

List of Subjects in 21 CFR Part 101

Food labeling, Nutrition, Reporting and recordkeeping requirements.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, 21 CFR part 101 is amended as follows:

PART 101—FOOD LABELING

■ 1. The authority citation for part 101 continues to read as follows:

Authority: 15 U.S.C. 1453, 1454, 1455; 21 U.S.C. 321, 331, 342, 343, 348, 371; 42 U.S.C. 243, 264, 271.

■ 2. Section 101.75 is amended by revising paragraphs (c)(1) and (c)(2)(ii) to read as follows:

§ 101.75 Health claims: dietary saturated fat and cholesterol and risk of coronary heart disease.

* * * * *

(c) * * *

(1) All requirements set forth in § 101.14 shall be met, except § 101.14(e)(6) with respect to a raw fruit or vegetable.

(2) * * *

(ii) *Nature of the food.* (A) The food shall meet all of the nutrient content requirements of § 101.62 for a "low saturated fat" and "low cholesterol" food.

(B) The food shall meet the nutrient content requirements of § 101.62 for a "low fat" food, unless it is a raw fruit or vegetable; except that fish and game meats (*i.e.*, deer, bison, rabbit, quail, wild turkey, geese, and ostrich) may meet the requirements for "extra lean" in § 101.62.

* * * * *

Dated: December 9, 2016.

Leslie Kux,

Associate Commissioner for Policy.

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BILLING CODE 4164–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Parts 878, 880, and 895

[Docket No. FDA–2015–N–5017]

RIN 0910–AH02

Banned Devices; Powdered Surgeon's Gloves, Powdered Patient Examination Gloves, and Absorbable Powder for Lubricating a Surgeon's Glove

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA or Agency) has determined that Powdered Surgeon's Gloves, Powdered Patient Examination Gloves, and Absorbable Powder for Lubricating a Surgeon's Glove present an unreasonable and substantial risk of illness or injury and that the risk cannot be corrected or eliminated by labeling or a change in labeling. Consequently, FDA is banning these devices.

DATES: This rule is effective on January 18, 2017.

ADDRESSES: For access to the docket to read background documents or comments received, go to <https://www.regulations.gov> and insert the docket number found in brackets in the

heading of this final rule into the "Search" box and follow the prompts, and/or go to the Division of Dockets Management, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852.

FOR FURTHER INFORMATION CONTACT:

Michael J. Ryan, Center for Devices and Radiological Health, Food and Drug Administration, 10903 New Hampshire Ave., Bldg. 66, Rm. 1615, Silver Spring, MD 20993, 301-796-6283, email: michael.ryan@fda.hhs.gov.

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I. Executive Summary

A. Purpose and Coverage of the Final Rule

Medical gloves play a significant role in the protection of both patients and health care personnel in the United States. Health care personnel rely on medical gloves as barriers against transmission of infectious diseases and contaminants when conducting surgery, as well as when conducting more limited interactions with patients. Various types of powder have been used to lubricate gloves so that wearers could don the gloves more easily. However, the use of powder on medical gloves presents numerous risks to patients and health care workers, including inflammation, granulomas, and respiratory allergic reactions.

A thorough review of all currently available information supports FDA's

conclusion that powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove should be banned. FDA has concluded that the risks posed by powdered gloves, including health care worker and patient sensitization to natural rubber latex (NRL) allergens, surgical complications related to peritoneal adhesions, and other adverse health events not necessarily related to surgery, such as inflammatory responses to glove powder, are important, material, and significant in relation to the benefit to public health from their continued marketing. FDA has carefully evaluated the risks and benefits of powdered gloves and the risks and benefits of the state of the art, which includes viable non-powdered alternatives that do not carry any of the risks associated with glove powder, and has determined that the risk of illness or injury posed by powdered gloves is unreasonable and substantial. Further, FDA believes that this ban would likely have minimal economic and shortage impact on the health care industry. Thus, a transition to alternatives in the marketplace should not result in any detriment to public health.

This rule applies to powdered patient examination gloves, powdered surgeon's gloves, and absorbable powder for lubricating a surgeon's glove. This includes all powdered medical gloves except powdered radiographic protection gloves. Because we are not aware of any powdered radiographic protection gloves that are currently on the market, FDA lacks the evidence to determine whether the banning standard would be met for this particular device. The ban does not apply to powder used in the manufacturing process (e.g., former-release powder) of non-powdered gloves, where that powder is not intended to be part of the final finished glove. Finished non-powdered gloves are expected to include no more than trace amounts of residual powder from these processes, and the Agency encourages manufacturers to ensure finished non-powdered gloves have as little powder as possible. In our 2008 Medical Glove Guidance Manual (Ref. 1), we recommended that non-powdered gloves have no more than 2 milligrams (mg) of residual powder and debris per glove, as determined by the Association for Testing and Materials (ASTM) D6124 test method (Ref. 2). The Agency continues to believe this amount is an appropriate maximum level of residual powder. The ban also does not apply to powder intended for use in or on other

medical devices, such as condoms. FDA has not seen evidence that powder intended for use in or on other medical devices, such as condoms, presents the same public health risks as that on powdered medical gloves.

B. Summary of the Major Provisions of the Final Rule

In this final rule, FDA is banning the following devices: (1) Powdered surgeon's gloves, (2) powdered patient examination gloves, and (3) absorbable powder for lubricating a surgeon's glove. Because the classification regulations for these device types do not distinguish between powdered and non-powdered versions, FDA is amending the descriptions of these devices in the regulations to specify that the regulations for patient examination and surgeon's gloves will apply only to non-powdered gloves while the powdered version of each type of glove will be added to the listing of banned devices in the regulations.

Many comments requested that FDA revise the scope of the ban to include all NRL gloves. Many comments from industry requested that the proposed effective date be extended beyond 30 days after the date of publication of the final rule. Of the comments that do not support the ban, commenters noted the need for powdered gloves to aid in donning gloves and tactile sense and the reduced risks associated with current powdered gloves that have less powder. The remaining comments are not clearly in support or opposition to the proposal.

C. Legal Authority

Powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove are defined as devices under section 201(h) of the Federal Food, Drug, and Cosmetic Act (the FD&C Act) (21 U.S.C. 321(h)). Section 516 of the FD&C Act (21 U.S.C. 360f) authorizes FDA to ban a device if it finds, on the basis of all available data and information, that the device presents substantial deception or unreasonable and substantial risks of illness or injury, which cannot be corrected by labeling or a change in labeling. This rule amends 21 CFR 878.4460, 878.4480, 880.6250, 895.102, 895.103, and 895.104. FDA's legal authority to modify §§ 878.4460, 878.4480, 880.6250, 895.102, 895.103, and 895.104 arises from the device and general administrative provisions of the FD&C Act (21 U.S.C. 352, 360f, 360h, 360i, and 371).

D. Costs and Benefits

The final rule is expected to provide a positive net benefit (estimated benefits minus estimated costs) to society. Banning powdered glove products is not expected to impose any costs to society, but is expected to reduce the number of adverse events associated with using powdered gloves. The primary public health benefit from adoption of the rule would be the value of the reduction in adverse events associated with using powdered gloves. The Agency estimates maximum total annual net benefits to range between \$26.8 million and \$31.8 million.

II. Background

A. Need for the Regulation/History of the Rulemaking

On March 22, 2016, FDA issued a proposed rule to ban powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove (81 FR 15173). Section 516(a)(1) of the FD&C Act authorizes FDA to ban a device intended for human use by regulation if it finds, on the basis of all available data and information, that such a device "presents substantial deception or an unreasonable and substantial risk of illness or injury." For a more detailed discussion of the banning standard, we refer you to the preamble of the proposed rule. FDA issued the proposed regulation because it determined that powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove present an unreasonable and substantial risk of illness or injury and that the risk cannot be corrected or eliminated by labeling or a change in labeling.

The preamble to the proposed rule describes the history of powdered gloves and the citizen petitions received by the Agency that request a ban on powdered gloves. We refer readers to that preamble for information about the development of the proposed rule. The level and types of risk presented by powdered gloves varies depending on the composition and intended use of the glove. In aggregate, the risks of powdered gloves include severe airway inflammation, hypersensitivity reactions, allergic reactions (including asthma), allergic rhinitis, conjunctivitis, dyspnea, as well as granuloma and adhesion formation when exposed to internal tissue. We refer readers to the preamble of the proposed rule for details on the level and types of risks presented by powdered gloves. The benefits of powdered gloves appear to only include greater ease of donning

and doffing, decreased tackiness, and a degree of added comfort, which FDA believes are nominal when compared to the risks posed by these devices.

The state of the art of both surgeon's and patient examination gloves includes non-powdered alternatives that provide similar performance as the various powdered glove types do. That is, there are many non-powdered gloves available that have the same level of protection, dexterity, and performance. Thus, based on a careful evaluation of the risks and benefits of powdered gloves and the risks and benefits of the current state of the art, which includes readily available alternatives that carry none of the risks posed by powdered gloves, FDA has determined that the standard to ban powdered gloves has been met, and that it is appropriate to issue this ban.

Finally, as discussed in the proposed rule, FDA also determined the ban should apply to devices already in commercial distribution and devices already sold to the ultimate user, as well as to devices that would be sold or distributed in the future (see 21 CFR 895.21(d)(7)). This means that powdered gloves currently being used in the marketplace would be subject to this ban and adulterated under section 501(g) of the FD&C Act (21 U.S.C. 351(g)), and thus subject to enforcement action.

B. Summary of Comments to the Proposed Rule

The Agency requested public comments on the proposed rule, and the comment period closed on June 20, 2016. The Agency received approximately 100 comment letters on the proposed rule by the close of the comment period, each containing one or more comments on one or more issues. We received comments from a cross-section of patients and consumers, medical professionals, device manufacturers, and professional and trade associations. A majority of the comments supported the objectives of the rule in whole or in part, while a minority of the comments opposed the objectives of the rule. Some comments suggested changes to specific elements of the proposed rule or requested clarification of matters discussed in the proposed rule. See Section IV for the description of comments on the proposed rule and FDA's responses.

C. General Overview of the Final Rule

FDA published a proposed rule to ban powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove, because FDA

determined that these devices present an unreasonable and substantial risk of illness or injury and that the risk cannot be corrected or eliminated by labeling or a change in labeling (81 FR 15173).

In this final rule, FDA is banning the following devices: (1) Powdered surgeon's gloves (21 CFR 878.4460), (2) powdered patient examination gloves (21 CFR 880.6250), and (3) absorbable powder for lubricating a surgeon's glove (21 CFR 878.4480). Because the classification regulations for these device types do not distinguish between powdered and non-powdered versions, FDA is amending the descriptions of these devices in the regulations to specify that the regulations for surgeon's gloves (21 CFR 878.4460) and patient examination gloves (21 CFR 880.6250) will apply only to non-powdered gloves while the powdered version of each type of glove will be added to 21 CFR part 895, subpart B—Listing of Banned Devices.

D. Clarifying Changes to the Rule

While FDA believes that the preamble to the proposed rule was clear that the proposed ban would apply to all powdered surgeon's gloves and all powdered patient examination gloves, in reviewing the terminology used in the proposed additions to 21 CFR part 895, FDA determined that term "synthetic latex" would not cover every type of non-NRL material that is used to manufacture powdered gloves. It was not FDA's intent to limit the ban to only powdered NRL and powdered synthetic latex gloves, and we believe that this intent was clear from the content of the preamble to the proposed rule, which stated that the ban "would apply to all powdered gloves except powdered radiographic protection gloves." As such, FDA has now revised the identification in this final rule to clarify that the ban applies to all powdered surgeon's gloves and powdered patient examination gloves without reference to the type of material from which they are made. Additionally, the identification of non-powdered surgeon's gloves and non-powdered patient examination gloves is also being revised to remove reference to material.

III. Legal Authority

Powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove are defined as medical devices under section 201(h) of the FD&C Act (21 U.S.C. 321). Section 516 of the FD&C Act (21 U.S.C. 360f) authorizes FDA to ban a device if it finds, on the basis of all available data and information, that the device

presents substantial deception or unreasonable and substantial risks of illness or injury, which cannot be corrected by labeling or a change in labeling. This rule amends §§ 878.4460, 878.4480, 880.6250, 895.102, 895.103, and 895.104. FDA's legal authority to modify §§ 878.4460, 878.4480, 880.6250, 895.102, 895.103, and 895.104 arises from the device and general administrative provisions of the FD&C Act (21 U.S.C. 352, 360f, 360h, 360i, and 371).

IV. Comments on the Proposed Rule and FDA's Responses

A. Introduction

We received approximately 100 comment letters on the proposed rule by the close of the comment period, each containing one or more comments on one or more issues. We received comments from a cross-section of patients and consumers, medical professionals, device manufacturers, and professional and trade associations. A majority of the comments supported the objectives of the rule in whole or in part, while a minority of the comments opposed the objectives of the rule. Some comments suggested changes to specific elements of the proposed rule or requested clarification of matters discussed in the proposed rule.

We describe and respond to the comments in section IV.B through E. We have numbered each comment to help distinguish between different comments. We have grouped similar comments together under the same number, and, in some cases, we have separated different issues discussed in the same comment and designated them as distinct comments for purposes of our responses. The number assigned to each comment or comment topic is purely for organizational purposes and does not signify the comment's value or importance or the order in which comments were received.

B. Description of General Comments and FDA Response

Many comments made general remarks supporting or opposing the proposed rule without focusing on a particular proposed provision. In the following paragraphs, we discuss and respond to such general comments.

(Comment 1) Many comments support the proposed ban on powdered patient examination gloves and powdered surgeon's gloves. These comments from individual consumers, health care professionals, academia, and industry highlight several risks of the continued use of powdered gloves, including, among others, allergic reactions, post-

operative adhesions, and delayed wound healing.

(Response 1) FDA agrees with these comments. After further review of all available information and the comments submitted to the proposed rule, FDA has concluded that the public's exposure to the risks of powdered gloves is unreasonable and substantial in relation to the nominal public health benefit derived from the continued marketing of these devices, especially when considering the benefits and risks posed by readily available alternative devices. Therefore, FDA has determined that the standard for a ban on these devices has been met.

C. Description of Comments That Oppose the Regulation and FDA Response

FDA received some comments that oppose the proposed ban on powdered patient examination gloves and powdered surgeon's gloves for various reasons. We address each of these reasons for opposition in this section. After reviewing these comments, FDA has determined that the standard to ban powdered gloves has been met, and that it is appropriate to issue this ban. We are finalizing the ban with only clarifying changes.

(Comment 2) Comments oppose the proposed ban on powdered patient examination gloves and powdered surgeon's gloves because of difficulty donning or doffing non-powdered gloves. Two commenters specifically discuss hyperhidrosis with claims that it can add to the difficulty donning and doffing non-powdered gloves. One commenter has asserted that double-gloving is more difficult when using non-powdered gloves.

(Response 2) As described in the preamble of the proposed rule, we have concluded that the benefit of ease of donning or doffing powdered gloves is generally nominal (Ref. 3) in comparison to the risks posed by the continued marketing of powdered gloves, which, among others, include severe airway inflammation, hypersensitivity reactions, and allergic reactions (including asthma). Also, as noted in the proposed rule, a study of various brands of powdered and non-powdered NRL gloves by Cote et al. found that there are non-powdered latex gloves that are easily donned with wet or dry hands with relatively low force compared to the forces required to don powdered latex examination gloves (Ref. 3). Thus, FDA has considered ease of donning and doffing as a benefit as it applies within the banning standard, and has determined that the standard is met.

(Comment 3) Comments oppose the proposed ban on powdered patient examination gloves and powdered surgeon's gloves because of difficulty donning non-powdered gloves, leading to greater propensity of non-powdered gloves to tear. Some of these comments express concern that the reduced ability to separate the opening of a non-powdered glove or the greater propensity of non-powdered gloves to tear could potentially lead to a higher degree of contamination and post-procedure infections.

(Response 3) FDA disagrees with the assertion that non-powdered gloves have a higher propensity to tear and thus disagrees that use of non-powdered gloves presents a greater risk of contamination, post-procedure infections, or exposure of the user to blood. FDA does not believe there is compelling evidence to support the assertion that non-powdered gloves have a higher propensity to tear. Korniewicz, et al., determined that the presence of powder did not affect the durability of gloves or enhance glove donning (Ref. 4). Although Kerr, et al., identified a statistically significant difference in the durability of non-powdered vinyl gloves compared to powdered vinyl gloves, this difference may be attributed to glove type, manufacturer, and the fingernail length of users rather than the presence or absence of powder (Ref. 5). This study also found that vinyl gloves in general are less durable and have a greater propensity to tear compared to nitrile, neoprene, and latex gloves. Furthermore, as discussed in the response to comment 4, several studies have found that alternatives to non-powdered NRL gloves, such as nitrile and neoprene gloves, offer the same level of protection against contamination and exposure to blood as powdered NRL gloves (Refs. 5, 6, 7, 8, 9, and 10). Therefore, FDA has determined that suitable alternatives to powdered gloves are readily available in the marketplace.

(Comment 4) Commenters oppose the proposed ban on powdered patient examination gloves and powdered surgeon's gloves because the fit of powdered gloves is more comfortable than non-powdered gloves. Some of these comments assert that the reduced fit of non-powdered gloves inhibits the tactile sensation necessary to perform medical procedures.

(Response 4) FDA disagrees with the assertion that non-powdered gloves inhibit the tactile sensation necessary to perform medical procedures. The ban does not include non-powdered NRL gloves, which offer the same

performance characteristics of powdered NRL gloves, and several studies have found that alternatives, such as nitrile and neoprene gloves, offer the same level of protection, dexterity, and performance as NRL gloves (Refs. 5, 6, 7, 8, 9, and 10). Furthermore, the numerous risks posed by the continued marketing of powdered gloves outweigh the benefit of whatever additional level of comfort is provided from using powdered gloves instead of the non-powdered alternatives that carry none of these risks.

(Comment 5) Some comments oppose the proposed ban on powdered patient examination gloves and powdered surgeon's gloves, citing a lack of scientific evidence that gloves with reduced powder content, as those in use today, have the same risks as previously used gloves that had higher powder content.

(Response 5) FDA agrees that the maximum residual level of powder on powdered gloves is less than earlier types of powdered gloves. Historically, powdered medical gloves contained powder levels ranging from 50 to over 400 mg of powder per glove. Effective in 2002, the ASTM International recommended limits on powder levels is 15 mg per square decimeter for surgical gloves (ASTM D3577–2001) (Ref. 11) and 10 mg per square decimeter for patient examination gloves (ASTM D3578) (Ref. 12). As a result, FDA believes that gloves in use after 2002 follow these recommended limits and generally have lower powder content than earlier types of powdered gloves. Even so, several studies indicate that gloves with reduced powder levels continue to present unreasonable and substantial risks to patients and health care workers. For instance, a study conducted on the incidence of skin reactions for Greek endodontists from 2006 to 2012 found that glove powder accounted for the majority of skin reactions, and the replacement of powdered NRL gloves with non-powdered gloves resolved the majority of the adverse reactions (Ref. 13). Similarly, the risks of powdered gloves persist in non-clinical studies using gloves with reduced powder content, as demonstrated by the 2013 finding that surgeries performed with powdered gloves increased the number, density, and fibrotic properties of peritoneal adhesions in rats compared with surgeries performed with non-powdered gloves (Ref. 14). Also, the reduction in cases of NRL-induced occupational contact urticaria coincided with French hospitals transitioning to non-powdered gloves after 2004–2005 (Ref. 13).

Finally, FDA is not aware of any report in the literature that supports the assertion that currently marketed powdered gloves with lower powder content reduce the risks presented by powdered gloves (Ref. 15). In summary, FDA concludes that the risks of powder continue to be unreasonable and substantial for currently marketed powdered gloves despite lower powder content than previous generations of powdered gloves.

(Comment 6) Two comments oppose the proposed ban on powdered patient examination gloves and powdered surgeon's gloves, because the commenters believe a warning on the risks of powdered gloves is sufficient to mitigate the risks posed by these devices.

(Response 6) As described in Section IV of the proposed rule, FDA has determined that no change in labeling could correct the risk of illness or injury presented by the continued use of these devices. Powdered gloves have additional or increased risks to health compared to non-powdered gloves related to the spread of powder, and the fact that powder-transported contaminants such as NRL allergens can become aerosolized. Exposure to powder or latex allergens presents significant risks to health care workers and patients when inhaled or when exposed to internal tissue during oral, vaginal, gynecological, and rectal exams. Although labeling can raise awareness of these risks, we conclude that labeling cannot effectively mitigate these risks because it cannot prohibit the spread of glove powder or powder-transported contaminants. In addition, an important aspect of these devices is their ability to affect persons other than the individual who decides to wear or use them. For example, patients often do not know the type of gloves being worn by the health care professional treating them, but are still exposed to the potential dangers. Similarly, glove powder's ability to aerosolize and carry NRL proteins exposes individuals to harm via inhalation or surface contact. Thus, some of the risks posed by glove powder can impact persons completely unaware or unassociated with its employment and without the opportunity to consider the devices' labeling. Because of this inherent quality, adequate directions for use or warnings cannot be written that would provide reasonable assurance of the safe and effective use of these devices for all persons that might come in contact with them.

Due to the ability of powder to affect people who would not have an opportunity to read warning labels, and

because potential warning labels would raise awareness of the risks, but would not eliminate the risks posed by glove powder, FDA has determined no label or warning can correct the risks posed by these devices.

(Comment 7) One comment opposes the proposed ban on powdered patient examination gloves and powdered surgeon's gloves, because the solvent used to remove powder during the manufacture of non-powdered gloves may cause adverse reactions to the glove user.

(Response 7) FDA is not aware of any report in the literature that supports the assertion of widespread adverse reactions to solvent used in the manufacturing process. Non-powdered patient examination and surgeon's gloves require premarket notification (510(k)) submissions prior to marketing. During the review of these submissions, FDA evaluates the final finished glove, including manufacturing solvents that are present on the final glove. FDA recommends that manufacturers conduct and submit skin irritation and dermal sensitization studies in these submissions to evaluate potential issues with components, including manufacturing solvents (Ref. 1). Although individual hypersensitivity reactions to different materials may occur, FDA has been unable to find evidence in the literature of hypersensitivity to typical glove manufacturing materials other than glove powder or NRL. However, Palosuo, et al., reports that the use of hand sanitizers containing isopropyl alcohol prior to donning gloves could cause dermatitis reaction if the gloves are donned before the alcohol dries (Ref. 16). The occurrence of this reaction is unrelated to the manufacture of non-powdered gloves and unrelated to the use of non-powdered gloves as an alternative to powdered gloves. Given the lack of evidence of adverse reactions to solvents used in the manufacturing of non-powdered gloves, and the established evidence demonstrating the risks of powdered glove use, FDA continues to believe that powdered gloves and glove powder meet the banning standard.

(Comment 8) Several comments oppose the proposed ban on powdered patient examination gloves and powdered surgeon's gloves due to the expectation that users will ultimately have to pay more for medical gloves once the ban is finalized, because the cost of non-powdered gloves is currently higher than the cost of powdered gloves.

(Response 8) We do not find any evidence to support the claims that

current prices of non-powdered gloves are significantly higher than powdered gloves. As we stated in the preliminary regulatory impact analysis (PRIA), extensive searches of glove distributor pricing indicate that non-powdered gloves have become as affordable as powdered gloves. Our searches also revealed that the market is saturated with alternatives to powdered gloves, resulting in downward pressure on the prices of non-powdered gloves. In addition, the share of powdered medical gloves sales has been declining since at least 2000 while total sales of all disposable medical gloves have increased (Ref. 17). We would not expect this trend to be occurring without regulatory action if users of disposable medical gloves faced significantly higher prices for switching to non-powdered gloves. We therefore do not find it necessary to update our analysis based on these comments.

(Comment 9) We received one comment that disagrees with our determination that the availability of examination and surgical gloves would not be reduced.

(Response 9) We do not find any evidence to support these claims. As we stated in the PRIA, research shows only 7 percent of total sales of examination and surgical gloves to medical workers were projected to be from powdered gloves in 2010 (Ref. 17). Global Industry Analysts (GIA) projected the share of powdered disposable medical gloves sales to decrease to 2 percent in 2015, while total sales of all disposable medical gloves continue to increase (Ref. 17). We would not expect this trend to be occurring without regulatory action if there were a reduction in the availability of disposable examination and surgical gloves. We therefore do not find it necessary to update our analysis based on these comments.

(Comment 10) Commenters suggest there would be a loss in consumer utility due to the preference some medical workers may have for powdered gloves due to comfort and ease of use.

(Response 10) We stated in the PRIA that the remaining 7 percent continuing to use these powdered gloves may experience utility loss from the removal of powdered gloves from the market (Ref. 17). The potential loss in consumer utility would be due to the perceived loss in comfort from powdered gloves users switching to non-powdered gloves. However, as the GIA report shows, there has been a downward trend in total sales of powdered gloves since at least the year 2000 while total sales of all disposable medical gloves has increased (Ref. 17). We would not

expect this trend to be occurring without regulatory action if the loss in consumer utility to current medical workers were substantial. Korniewicz et al. reported no loss in consumer satisfaction in a sample of operating room staff switching to non-powdered surgical gloves (Ref. 4). We have not estimated this potential burden, but the evidence described here suggests that any burden would not be substantial. Further, even having considered that some degree of consumer comfort may be lost by banning powdered gloves, FDA continues to believe that this benefit is considerably outweighed by the numerous risks posed by powdered gloves.

(Comment 11) One comment opposes the proposed ban on powdered patient examination gloves and powdered surgeon's gloves, because the risks identified for powdered gloves are due to contaminants, such as pesticides and herbicides, in the powder that would not be present if the powder were manufactured in the United States.

(Response 11) FDA disagrees with the assertion that contaminated powder is the source of the risks identified for powdered gloves. FDA's proposal to ban powdered gloves and glove powder is based on various studies on the risks of powdered gloves due to the properties of the powder itself. Powdered gloves have additional or increased risks to health compared to non-powdered gloves. For example, powder on NRL gloves can aerosolize latex allergens, resulting in sensitization to latex and allergic reactions. Latex sensitization and allergic reactions are unrelated to any potential presence of manufacturing contaminants, such as pesticides and herbicides. Additional risks of powdered gloves include severe airway inflammation, conjunctivitis, dyspnea, as well as granuloma and adhesion formation when exposed to internal tissue. FDA's assessment of the available literature and information indicates that these risks are attributable to the powder itself, as opposed to any potential presence of manufacturing contaminants, such as pesticides and herbicides.

In addition, the powder used on powdered gloves is required to comply with FDA's Quality System regulation, which includes requirements for quality and inspection for the final finished gloves that protect against the introduction of contaminated devices into commerce. Among other requirements, device manufacturers must establish and maintain procedures to prevent contamination of equipment or product by substances that could reasonably be expected to have an

adverse effect on product quality (21 CFR 820.70(e)). FDA's Quality System regulation applies to gloves and glove powder sold in the United States, regardless of the manufacturing location.

D. Description of Comments on Scope of Ban and FDA Response

FDA received several comments requesting revision of the scope of the ban. The scope of the proposed ban includes powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove. The glove types include all powdered patient examination and surgeon's gloves, including NRL and synthetic latex gloves. In the following paragraphs, we discuss and respond to comments requesting revision of the scope of the ban. We are finalizing the ban without change to the scope, but clarifying that all powdered patient examination gloves and powder surgical gloves are banned, regardless of the material from which they are made.

(Comment 12) Several comments identify risks that result from the use of powdered and non-powdered NRL gloves. These comments request FDA to extend the ban to all NRL gloves, both powdered and non-powdered.

(Response 12) Unlike with powdered latex gloves, which have the ability to aerosolize glove powder and carry allergenic proteins, FDA believes the risk of allergic reaction to non-powdered NRL gloves, which affects the user and patients in direct contact with the glove, is adequately mitigated through already-required labeling that alerts users to this risk. NRL gloves must include a statement to alert users to the risk of allergic reactions caused by NRL (21 CFR 801.437). Further, several studies have indicated that the use of non-powdered NRL gloves reduces the risk of sensitization to allergenic NRL proteins and the number of allergic reactions experienced by those who are already sensitized (Refs. 18, 19, and 20). FDA believes that these study results, when considered alongside the risk mitigation that follows from FDA's required labeling for NRL products, demonstrates that non-powdered latex gloves can be safely used with appropriate caution for latex-sensitive patients and health care workers. Therefore, FDA has determined not to ban the use of all NRL gloves.

(Comment 13) Several comments raise the issue of life threatening latex allergy events that result from various uses of NRL gloves including food preparation and food service. Several of these comments assert that the Agency should broaden the scope of the ban to cover all

NRL gloves for all uses including food preparation and food service.

(Response 13) We have concluded that it is not appropriate to address a proposal to ban gloves used for food preparation because these gloves do not meet the definition of a device under section 201(h) of the FD&C Act and are thus not subject to section 516 of the FD&C Act (21 U.S.C. 360f), which provides the statutory authority to ban devices within FDA's authority to regulate such products.

(Comment 14) One comment asserts that the ban on powdered gloves should not apply to dental practice, because the risks are not applicable to dental practice.

(Response 14) FDA disagrees with the assertion that the risks of powdered gloves are not applicable to dental practice. Dentists and dental patients face the same risks as other medical practices in terms of the potential for powder exposure to open cavities or open wounds, and for powder, if used with NRL gloves, to carry protein allergens. Several studies documenting the risks of powdered gloves in dental practices have been conducted, including Saary, et al., which identified that changing to low-protein and non-powdered NRL gloves reduced NRL allergy in dental students (Ref. 18). In addition, Charous et al., reported in 2000 that a dental office was able to reduce airborne NRL antigen levels to undetectable levels with the exclusive use of non-powdered NRL gloves, permitting a highly sensitized staff member to continue to work there (Ref. 21). These studies, among others (Refs. 13 and 22), indicate that the risks of powdered medical gloves apply to dental practice. Therefore, FDA has determined that the scope of the ban on powdered medical gloves should continue to include powdered gloves used in dental practice.

E. Description of Other Specific Comments and FDA Response

Many comments made specific remarks requesting clarification or revision to the proposed rule. In the following paragraphs, we discuss and respond to such specific comments.

(Comment 15) A number of comments request extension of the effective date of the ban. The proposed rule included a proposed effective date of 30 days after publication of the final rule for all devices, including those already in commercial distribution. The comments suggest a range of effective dates of 90 days to 18 months after publication of the final rule and assert that a longer transition period is necessary to allow

existing inventory to flow through the supply chain to providers and patients.

(Response 15) FDA is not extending the effective date of the ban for devices already in commercial distribution. We have concluded that powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's glove present an unreasonable and substantial risk of illness or injury and that the risk cannot be corrected or eliminated by labeling or a change in labeling. The continued marketing of these devices beyond the 30 day effective date would allow for the continued sale and purchase of devices that FDA has determined present an unreasonable and substantial risk to patients and health care workers. Therefore, FDA does not believe that it is in the best interest of the public health to extend the effective date for devices already in commercial distribution. In order to minimize the risk of continued exposure of health care workers and patients to these devices, the effective date for devices remains 30 days after the date of publication of this final rule.

(Comment 16) One comment requests that FDA not extend the effective date of the ban to allow companies to deplete their inventory of the devices.

(Response 16) As described in the response to comment 15, FDA agrees that it is in the best interest of the public health to not extend the effective date of the ban for devices already in commercial distribution. Therefore, the effective date of the ban for devices already in commercial distribution remains at 30 days after the date of publication of the final rule.

(Comment 17) A few comments request recommendations on the means of disposal or recycling of powdered gloves.

(Response 17) FDA recommends that unused inventories of powdered medical gloves remaining at domestic manufacturing and distribution locations be disposed of in accordance with standard industry practices. Unused supplies at hospitals, outpatient centers, clinics, medical and dental offices, other service delivery points (nursing homes, etc.), and in the possession of end users, will need to be disposed of according to established procedures of the local community's solid waste management system. Established procedures for these materials typically involve disposal in landfills or incineration. FDA has concluded that this final rule will not have a significant impact on the human environment. (See Section VII. Analysis of Environmental Impact.)

(Comment 18) One comment requests clarification on whether after the effective date of the ban the Agency will permit a manufacturer to export powdered medical gloves that are already physically located at distribution centers in the United States.

(Response 18) After the effective date of this final rule, manufacturers will not be allowed to import powdered medical gloves. However, while powdered medical gloves will be banned in the United States on the effective date of this final rule, manufacturers may export existing inventory of powdered gloves to a foreign country if the device complies with the laws of that country and has valid marketing authorization by the appropriate authority, as described in section 802 of the FD&C Act (21 U.S.C. 382)). If eligible for export under section 802 of the FD&C Act, a device intended for export will not be deemed adulterated or misbranded if it

(A) accords to the specifications of the foreign purchaser,

(B) is not in conflict with the laws of the country to which it is intended for export,

(C) is labeled on the outside of the shipping package that it is intended for export, and

(D) is not sold or offered for sale in domestic commerce.

V. Effective Date

This rule is effective January 18, 2017. The effective date of this rule applies to devices already in commercial distribution and those already sold to the ultimate user, as well as to devices that would be sold or distributed in the future. All powdered surgeon's gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon's gloves must be removed from the market upon the effective date of this final rule. Section 501(g) of the FD&C Act (21 U.S.C. 351(g)) deems a device to be adulterated if it is a banned device.

VI. Economic Analysis of Impacts

A. Introduction

We have examined the impacts of the final rule under Executive Order 12866, Executive Order 13563, the Regulatory Flexibility Act (5 U.S.C. 601–612), and the Unfunded Mandates Reform Act of 1995 (Pub. L. 104–4). Executive Orders 12866 and 13563 direct us to assess all costs and benefits of available regulatory alternatives and, when regulation is necessary, to select regulatory approaches that maximize net benefits (including potential economic, environmental, public health and safety,

and other advantages; distributive impacts; and equity). We have developed a comprehensive Economic Analysis of Impacts that assesses the impacts of the final rule. We believe that this final rule is not a significant regulatory action as defined by Executive Order 12866.

The Regulatory Flexibility Act requires us to analyze regulatory options that would minimize any significant impact of a rule on small entities. Because this rule imposes no new burdens, we certify that the final rule will not have a significant economic impact on a substantial number of small entities.

The Unfunded Mandates Reform Act of 1995 (section 202(a)) requires us to prepare a written statement, which includes an assessment of anticipated costs and benefits, before issuing “any rule that includes any Federal mandate that may result in the expenditure by State, local, and tribal governments, in the aggregate, or by the private sector, of \$100,000,000 or more (adjusted annually for inflation) in any one year.” The current threshold after adjustment for inflation is \$146 million, using the most current (2015) Implicit Price Deflator for the Gross Domestic Product. This final rule would not result in an expenditure in any year that meets or exceeds this amount.

B. Summary of Costs and Benefits

The final rule prohibits marketing of powdered surgeon’s gloves, powdered patient examination gloves, and absorbable powder for lubricating surgeon’s gloves. The rule does not cover or include powdered radiographic gloves.

The final rule is expected to provide a positive net benefit (estimated benefits minus estimated costs) to society. Banning powdered glove products is not expected to impose any costs to society. Extensive searches of glove distributor pricing indicate that improvements to non-powdered gloves have made these products as affordable as powdered gloves. The ban is expected to reduce the adverse events associated with using powdered gloves. The Agency estimates maximum total annual net benefits to range between \$26.8 million and \$31.8 million. The present discounted value of the estimated benefits over 10 years ranges from \$228.9 million to \$270.8 million at a 3 percent discount rate and from \$188.5 million to \$223 million at a 7 percent discount rate.

FDA has examined the economic implications of the rule as required by the Regulatory Flexibility Act. If a rule will have a significant economic impact on a substantial number of small

entities, the Regulatory Flexibility Act requires us to analyze regulatory options that would lessen the economic effect of the rule on small entities. This rule will not impose any new burdens on small entities, and thus will not impose a significant economic impact on a substantial number of small entities.

The full discussion of the economic impacts of the rule, which includes a list of changes made in the final regulatory impact analysis, in accordance with Executive Order 12866, Executive Order 13563, the Regulatory Flexibility Act, and the Unfunded Mandates Reform Act is available at <https://www.regulations.gov> under the docket number (FDA–2015–N–5017) for this rule and at <http://www.fda.gov/AboutFDA/ReportsManualsForms/Reports/EconomicAnalyses/default.htm#> (Ref. 23).

VII. Analysis of Environmental Impact

FDA has carefully considered the potential environmental effects of this final rule and of possible alternative actions. In doing so, the Agency focused on the environmental impacts of its action as a result of disposal of unused powdered surgeon’s gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon’s glove that will need to be handled after the rule is finalized.

The environmental assessment (EA) considered each of the alternatives in terms of the need to provide maximum reasonable protection of human health without resulting in a significant impact on the environment. The EA considered environmental impacts related to landfill and incineration of solid waste at municipal solid waste (MSW) facilities nationwide. The selected action, if finalized, will result in an initial batch disposal of unused powdered surgeon’s gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon’s glove from user facilities to MSW facilities nationwide, followed by a rapid decrease in the rate of disposal of these devices, as supplies are depleted. The selected action does not change the ultimate disposition of these devices but expedites their rate of disposal and ceases future production. Overall, given the limited number of powdered surgeon’s gloves, powdered patient examination gloves, and absorbable powder for lubricating a surgeon’s glove, currently in commercial distribution, the selected action is expected to have no significant impact on MSW and landfill facilities and the environment in affected communities.

The Agency has carefully considered the potential environmental effects of this action. FDA has concluded that the action will not have a significant impact on the human environment, and that an environmental impact statement is not required. The Agency’s finding of no significant impact and the evidence supporting that finding, contained in an EA, may be seen in the Division of Dockets Management (see **ADDRESSES**) between 9 a.m. and 4 p.m., Monday through Friday (Ref. 24).

VIII. Paperwork Reduction Act of 1995

This final rule contains no collection of information. Therefore, FDA is not required to seek clearance by Office of Management and Budget under the Paperwork Reduction Act of 1995.

IX. Federalism

We have analyzed this final rule in accordance with the principles set forth in Executive Order 13132. FDA has determined that the rule does not contain policies that have substantial direct effects on the States, on the relationship between the National Government and the States, or on the distribution of power and responsibilities among the various levels of government. Accordingly, we conclude that the rule does not contain policies that have federalism implications as defined in the Executive order and, consequently, a federalism summary impact statement is not required.

X. References

The following references are on display in the Division of Dockets Management (see **ADDRESSES**) and are available for viewing by interested persons between 9 a.m. and 4 p.m., Monday through Friday; they are also available electronically at <https://www.regulations.gov>. FDA has verified the Web site addresses, as of the date this document publishes in the **Federal Register**, but Web sites are subject to change over time.

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List of Subjects

21 CFR Parts 878 and 880

Medical devices.

21 CFR Part 895

Administrative practice and procedure, Labeling, Medical devices.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, 21 CFR parts 878, 880, and 895 are amended as follows:

PART 878—GENERAL AND PLASTIC SURGERY DEVICES

■ 1. The authority citation for part 878 continues to read as follows:

Authority: 21 U.S.C. 351, 360, 360c, 360e, 360j, 360l, 371.

■ 2. Amend § 878.4460 by revising the section heading and paragraph (a) to read as follows:

§ 878.4460 Non-powdered surgeon's glove.

(a) *Identification.* A non-powdered surgeon's glove is a device intended to be worn on the hands of operating room personnel to protect a surgical wound from contamination. A non-powdered surgeon's glove does not incorporate powder for purposes other than manufacturing. The final finished glove includes only residual powder from manufacturing.

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§ 878.4480 [Removed]

■ 3. Remove § 878.4480.

PART 880—GENERAL HOSPITAL AND PERSONAL USE DEVICES

■ 4. The authority citation for part 880 continues to read as follows:

Authority: 21 U.S.C. 351, 360, 360c, 360e, 360j, 371.

■ 5. Amend § 880.6250 by revising the section heading and paragraph (a) to read as follows:

§ 880.6250 Non-powdered patient examination glove.

(a) *Identification.* A non-powdered patient examination glove is a disposable device intended for medical purposes that is worn on the examiner's hand or finger to prevent contamination between patient and examiner. A non-powdered patient examination glove does not incorporate powder for purposes other than manufacturing. The final finished glove includes only residual powder from manufacturing.

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PART 895—BANNED DEVICES

■ 6. The authority citation for part 895 continues to read as follows:

Authority: 21 U.S.C. 352, 360f, 360h, 360i, 371.

■ 7. Add § 895.102 to read as follows:

§ 895.102 Powdered surgeon's glove.

(a) *Identification.* A powdered surgeon's glove is a device intended to be worn on the hands of operating room personnel to protect a surgical wound from contamination. A powdered surgeon's glove incorporates powder for purposes other than manufacturing.

(b) [Reserved]

■ 8. Add § 895.103 to read as follows:

§ 895.103 Powdered patient examination glove.

(a) *Identification.* A powdered patient examination glove is a disposable device intended for medical purposes that is worn on the examiner's hand or finger to prevent contamination between patient and examiner. A powdered patient examination glove incorporates powder for purposes other than manufacturing.

(b) [Reserved]

■ 9. Add § 895.104 to read as follows:

§ 895.104 Absorbable powder for lubricating a surgeon's glove.

Absorbable powder for lubricating a surgeon's glove is a powder made from cornstarch that meets the specifications for absorbable powder in the United States Pharmacopeia (U.S.P.) and that is intended to be used to lubricate the surgeon's hand before putting on a surgeon's glove. The device is absorbable through biological degradation.

Dated: December 13, 2016.

Leslie Kux,

Associate Commissioner for Policy.

FR Doc 20 6-30382 Fi ed 2- 6- 6 8 45 a]

BILLING CODE 4164-01-P

DEPARTMENT OF HEALTH AND HUMAN SERVICES**Food and Drug Administration****21 CFR Part 880**

[Docket No. FDA-2015-N-0701]

General Hospital and Personal Use Devices: Renaming of Pediatric Hospital Bed Classification and Designation of Special Controls for Pediatric Medical Crib; Classification of Medical Bassinet

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is issuing a final rule to rename pediatric hospital beds as pediatric medical cribs and establish special controls for these devices. FDA is also establishing a separate classification regulation for medical bassinets, previously under the pediatric hospital bed classification regulation, as a class II (special controls) device. In addition, this rule continues to allow both devices to be exempt from premarket notification and use of the device in traditional health care settings and permits prescription use of pediatric medical cribs and bassinets outside of traditional health care settings.

DATES: This order is effective on January 18, 2017.

FOR FURTHER INFORMATION CONTACT:

Michael J. Ryan, Center for Devices and Radiological Health, Food and Drug Administration, 10903 New Hampshire Ave., Bldg. 66, Rm. 1615, Silver Spring, MD 20993-0002, 301-796-6283.

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I. Executive Summary**A. Purpose and Coverage of the Final Rule**

Pediatric medical cribs that meet the definition of a device in section 201(h) of the Federal Food, Drug, and Cosmetic Act (the FD&C Act) (21 U.S.C. 321(h)) (referred to as pediatric medical cribs or cribs intended for medical purposes) (product code FMS) are regulated by FDA and will have to comply with the special controls identified in this rule for pediatric medical cribs. Cribs that do not meet the device definition (referred

to as cribs for non-medical purposes) must meet the Consumer Product Safety Commission's (CPSC's) regulations and guidelines.

In the **Federal Register** of December 28, 2010 (75 FR 81766), the CPSC issued a final rule prohibiting the use of the drop-side rail design for non-medical cribs in consumer households as of June 28, 2011. CPSC's rule established new standards for full-size and non-full-size cribs intended for non-medical purposes, which effectively prohibited the manufacture or sale of cribs intended for non-medical purposes with a drop-side rail design in households, child care facilities, family child care homes, and places of public accommodation. This rule did not affect pediatric medical cribs regulated by FDA, which typically contain a drop-side rail design that includes movable and latchable side and end rails. Although drop-side cribs intended for non-medical purposes are now prohibited, there is still a need for pediatric medical cribs with drop-side rails inside and outside of traditional health care settings. Pediatric medical cribs with drop-side rails are extremely helpful for patient care in hospital settings and even outside of traditional health care settings, such as day care centers caring for infants and children with disabilities, because they allow parents and care givers easy access to children to perform routine and emergency medical procedures, including, but not limited to, cardiopulmonary resuscitation (CPR), blood collection, intravenous (IV) insertion, respiratory care, and skin care. These drop-side rail cribs also make it easier for hospital staff to facilitate safe patient transport and reduce the chance of care giver injury.

Over the last 5 years, FDA has received over 500 adverse event reports, or Medical Device Reports (MDRs), associated with open pediatric medical cribs, through the Agency's Manufacturer and User Facility Device Experience (MAUDE) database. There were adverse event reports of serious injuries, including reports of entrapment, which were predominantly entrapments of extremities (legs or arms). The majority of MDRs for medical cribs were for malfunctions such as drop-side rails not latching or lowering, brakes not holding, wheels or casters breaking, and where applicable, scales not reading correct weights. As a result of the risks to health and need for continued use of pediatric medical cribs in traditional health care settings and non-traditional settings, FDA is revising the identification for § 880.5140 (21 CFR 880.5140) to include only pediatric

Exhibit 102

NTP TECHNICAL REPORT
ON THE
TOXICOLOGY AND CARCINOGENESIS
STUDIES OF TALC
(CAS NO. 14807-96-6)
IN F344/N RATS AND B6C3F₁ MICE
(INHALATION STUDIES)

Scheduled Peer Review Date: June 23-24, 1992

NOTICE

This is a DRAFT Technical Report prepared for public review and comment. Until this DRAFT has been reviewed and approved by the NTP Board of Scientific Counselors' Technical Reports Review Subcommittee in public session, the interpretations described herein do not represent the official scientific position of the National Toxicology Program. Following peer review, readers should contact NTP for the final version of this Technical Report.

NTP TR-421

NIH Publication No. 92-3152

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

**Plaintiff's Exhibit
No.**

P-11

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ABSTRACT

TALC (Non-Asbestiform)

CAS No. 14807-96-6

Molecular Formula: $Mg_3Si_4O_{10}(OH)_2$ Molecular Weight: 379.26

Synonyms: Talcum; Agalite; Emul 596; non-asbestiform talc; non-fibrous talc; Steatite; hydrous magnesium silicate.

Talc ore may contain several other minerals including calcite, dolomite, magnesite, tremolite, anthophyllite, antigorite, quartz, pyrophyllite, micas, or chlorites. Since talc products are sold in a multitude of grades which have physical or functional characteristics especially suited for particular applications, occupational and consumer exposures to talc are complex. Recently, epidemiology studies have revealed an association between non-fibrous talc and lung cancer risk. Talc was nominated by NIOSH for study by the NTP because of widespread human exposure and because of the lack of adequate information on its chronic toxicity and potential carcinogenicity. Toxicology and carcinogenicity studies of talc (non-asbestiform, cosmetic grade), a finely powdered hydrous magnesium silicate, were conducted by exposing groups of F344/N rats to aerosols for 6 hours daily, 5 days per week for up to 113 weeks for males and 122 weeks for females. Groups of B6C3F₁ mice were exposed similarly for up to 103 or 104 weeks.

LIFETIME STUDY IN RATS

Groups of 50 male and 49 or 50 female rats were exposed to aerosols containing 0, 6, or 18 mg/m³ talc until mortality in any exposure group reached 80% (113 weeks for males and 122 weeks for females). In a special study, additional groups of 22 male and 22 female rats were similarly exposed and examined for interim pathology evaluations or pulmonary function tests after 6, 11, 18, and 24 months and lung biochemistry and cytology studies after 24 months. The talc aerosols had a median mass aerodynamic diameter of 2.7 μ m in the 6 mg/m³ chamber and a median diameter of 3.2 μ m in the 18 mg/m³ chamber with geometric standard deviations of 1.9 μ m. However, there was a 7-week period beginning at study week 11 during which the

chamber concentration for the 18 mg/m³ rats varied from approximately 30 to 40 mg/m³ because of difficulties with the aerosol concentration monitoring system. Further, there was a 12-week period beginning at approximately week 70 during which there were difficulties in generating the talc aerosol, and the chamber concentrations for rats and mice were substantially lower than the target concentrations.

Survival, Body Weights, and Clinical Findings

The survival of male and female rats exposed to talc was similar to that of the controls. Mean body weights of rats exposed to 18 mg/m³ were slightly lower than those of controls after week 65. No clinical findings were attributed to talc exposure.

Pathology Findings

Absolute and relative lung weights of male rats exposed to 18 mg/m³ were significantly greater than those of controls at the 6-, 11-, and 18-month interim evaluations and at the end of the lifetime study, while those of female rats exposed to 18 mg/m³ were significantly greater at the 11-, 18-, and 24-month interim evaluations and at the end of the lifetime study. Inhalation exposure of rats to talc produced a spectrum of inflammatory, reparative, and proliferative processes in the lungs. Granulomatous inflammation occurred in nearly all exposed rats and the severity increased with exposure duration and concentration. Hyperplasia of the alveolar epithelium and interstitial fibrosis occurred in or near foci of inflammation in many exposed rats, while squamous metaplasia of the alveolar epithelium and squamous cysts were also occasionally seen. Accumulations of macrophages (histiocytes), most containing talc particles, were found in the peribronchial lymphoid tissue of the lung and in the bronchial and mediastinal lymph nodes. In female rats, the incidences of alveolar/bronchiolar

adenoma, carcinoma, and adenoma or carcinoma (combined) in the 18 mg/m³ group were significantly greater than those of controls. The incidences of pulmonary neoplasms in exposed groups of male rats were similar to those in controls.

Minor alterations attributed to talc exposure were also seen in the upper respiratory tract. Hyperplasia of the respiratory epithelium of the nasal mucosa in males and accumulation of cytoplasmic, eosinophilic droplets in the nasal mucosal epithelium in male and female rats occurred with a concentration-related increased incidence in the exposed groups.

Adrenal medulla pheochromocytomas (benign, malignant, or complex combined) occurred with a significant positive trend in male and female rats, and the incidences in the 18 mg/m³ groups were significantly greater than those of controls. Although adrenal medulla hyperplasia occurred with similar frequency among exposed and control females, the incidences of hyperplasia in exposed males were significantly lower than in controls.

Lung Talc Burden

Lung talc burdens of male and female rats exposed to 6 mg/m³ were similar and increased progressively from 6 to 24 months. Lung talc burdens of females exposed to 18 mg/m³ also increased progressively from 6 to 24 months, while those of males exposed to 18 mg/m³ remained about the same after 18 months. Lung burdens were generally proportional to exposure concentration at each interim evaluation.

Pulmonary Function, Bronchoalveolar Lavage, and Lung Biochemistry

In exposed male and female rats there was a concentration-related impairment of respiratory function which increased in severity with increasing exposure duration. The impairment was characterized by reductions in lung volume (total lung capacity, vital capacity, and forced vital capacity), lung compliance, gas exchange efficiency (carbon monoxide diffusing capacity), and nonuniform intrapulmonary gas distribution.

After 24 months, rats exposed to talc had significant increases in total protein, β -glucuronidase, lactate dehydrogenase, alkaline phosphatase, and polymorphonuclear leukocytes in bronchoalveolar lavage fluid. Viability and phagocytic activity of macrophages recovered from lavage fluid were not affected by talc exposure.

Total lung collagen was significantly increased in rats at both exposure concentrations after 24 months, while collagenous peptides in lavage fluid and percent newly synthesized protein from females, but not males, were also significantly increased at the 6 or 18 mg/m³ levels. In addition, lung proteinase activity, primarily cathepsin D-like activity, was significantly greater in exposed males and females. Rats exposed to talc also had significant increases in collagenous peptides and acid proteinase in lung homogenates.

2-YEAR STUDY IN MICE

Groups of 47 to 49 male and 48 to 50 female mice were exposed to aerosols containing 0, 6, or 18 mg/m³ talc for up to 103 or 104 weeks. In a special study, additional groups of 39 or 40 male and 40 female mice similarly exposed were examined for interim pathology evaluations, lung biochemistry, and cytology studies after 6, 12, and 18 months of exposure. The talc aerosols had a median mass aerodynamic diameter of 3.3 μ m with a geometric standard deviation of 1.9 μ m in the 6 mg/m³ chamber, and a median diameter of 3.6 μ m with a geometric standard deviation of 2.0 μ m in the 18 mg/m³ chamber.

Survival, Body Weights, and Clinical Findings

Final mean body weights and survival of male and female mice exposed to talc were similar to those of the controls. There were no clinical findings attributed to talc exposure.

Pathology Findings

Inhalation exposure of mice to talc was associated with chronic active inflammation and the accumulation of macrophages in the lung. In contrast to rats, hyperplasia of the alveolar epithelium, squamous metaplasia, or interstitial fibrosis were not associated with the inflammatory response in mice, and the incidences of pulmonary neoplasms in exposed and control groups of mice were similar. Accumulations of macrophages (histiocytes) containing talc particles were also present in the bronchial lymph node.

In the upper respiratory tract, cytoplasmic alteration, consisting of the accumulation of cytoplasmic eosinophilic droplets in the nasal mucosal epithelium, occurred with a concentration-related increased incidence in exposed male and female mice.

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Lung Talc Burden

Lung talc burdens of mice exposed to 6 mg/m³ were similar between males and females and increased progressively from 6 to 24 months, except for males at 18 months. The lung talc burdens of mice exposed to 18 mg/m³ were also similar between the sexes at each interim evaluation. Although the talc burdens of males and females increased substantially from 6 to 24 months, the values at 12 and 18 months were similar. Generally, lung burdens of mice exposed to 18 mg/m³ were disproportionately greater than those of mice exposed to 6 mg/m³, suggesting that clearance of talc from the lung was impaired, or impaired to a greater extent, in mice exposed to 18 mg/m³ than in mice exposed to 6 mg/m³.

Bronchoalveolar Lavage and Lung Biochemistry

Increases in total protein, β -glucuronidase, lactate dehydrogenase, and glutathione reductase, total nucleated cells, and polymorphonuclear leukocytes in bronchoalveolar lavage fluid were observed primarily in mice exposed to 18 mg/m³, although some parameters were also increased in mice exposed to 6 mg/m³.

The amount of collagenous peptides in lavage fluid and total lung collagen were increased in male and female mice exposed to 18 mg/m³. Acid proteinase activity, principally cathepsin D-like activity, of lung homogenate supernatant fluid was also significantly

increased in mice at the 18 mg/m³ exposure concentration.

CONCLUSIONS

Under the conditions of these inhalation studies, there was *some evidence of carcinogenic activity** of talc in male F344/N rats based on an increased incidence of benign and malignant pheochromocytomas of the adrenal gland. There was *clear evidence of carcinogenic activity* of talc in female F344/N rats based on increased incidences of alveolar/bronchiolar adenomas and carcinomas of the lung and benign and malignant pheochromocytomas of the adrenal gland. There was *no evidence of carcinogenic activity* of talc in male or female B6C3F₁ mice exposed to 6 or 18 mg/m³.

The principal toxic lesions associated with inhalation exposure to talc in rats included chronic granulomatous inflammation, alveolar epithelial hyperplasia, squamous metaplasia and squamous cysts, and interstitial fibrosis of the lung. These lesions were accompanied by impaired pulmonary function characterized primarily by reduced lung volumes, reduced dynamic and/or quasistatic lung compliance, reduced gas exchange efficiency, and nonuniform intrapulmonary gas distribution. In mice, inhalation exposure to talc produced chronic inflammation of the lung with the accumulation of alveolar macrophages.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9.

Summary of the Lifetime and 2-Year Carcinogenicity Studies of Talc

	Male F344/N Rats	Female F344/N Rats	Male B6C3F ₁ Mice	Female B6C3F ₁ Mice
Exposure levels	0, 6, or 18 mg/m ³	0, 6, or 18 mg/m ³	0, 6, or 18 mg/m ³	0, 6, or 18 mg/m ³
Body weights	High-dose group slightly lower than controls	High-dose group slightly lower than controls	Exposed groups similar to controls	Exposed groups similar to controls
Survival rates	9/50, 14/50, 16/50	11/50, 13/49, 9/50	30/47, 28/48, 32/49	30/49, 23/48, 25/50
Neoplastic effects	Adrenal medulla: benign or malignant pheochromocytoma (26/49, 32/48, 37/47)	Lung: alveolar/bronchiolar adenoma (1/50, 0/48, 9/50); alveolar/bronchiolar carcinoma (0/50, 0/48, 5/50); alveolar/bronchiolar adenoma or carcinoma (1/50, 0/48, 13/50) Adrenal medulla: benign or malignant pheochromocytoma (13/48, 14/47, 23/49)	None	None
Nonneoplastic effects	Lung: granulomatous inflammation (2/49, 50/50, 49/50); interstitial fibrosis (1/49, 16/50, 33/50); alveolar epithelial hyperplasia (5/49, 26/50, 38/50); peribronchial hyperplasia (0/49, 12/50, 8/50); cyst (0/49, 0/50, 3/50); alveolar squamous metaplasia (0/49, 0/50, 2/50) Lymph node (bronchial): histiocytic hyperplasia (0/41, 44/48, 46/49); (mediastinal) histiocytic hyperplasia (0/48, 40/49, 43/47) Nose: respiratory epithelial hyperplasia (0/49, 3/48, 14/47); cytoplasmic alteration (3/49, 18/48, 40/47)	Lung: granulomatous inflammation (2/50, 47/48, 50/50); interstitial fibrosis (1/50, 24/48, 44/50); alveolar epithelial hyperplasia (2/50, 27/48, 47/50); peribronchial hyperplasia (0/50, 8/48, 9/50); cyst (0/50, 1/48, 7/50); alveolar squamous metaplasia (0/50, 0/48, 8/50) Lymph node (bronchial): histiocytic hyperplasia (0/46, 40/47, 45/47); (mediastinal) histiocytic hyperplasia (0/47, 33/44, 40/47) Nose: cytoplasmic alteration (5/48, 23/45, 46/48)	Lung: chronic inflammation (0/45, 16/47, 40/48); macrophage hyperplasia (3/45, 46/47, 48/48) Lymph node (bronchial): histiocytic hyperplasia (1/32, 32/39, 42/44) Nose: cytoplasmic alteration (5/45, 23/46, 40/47)	Lung: chronic inflammation (0/46, 25/48, 38/50); macrophage hyperplasia (2/46, 45/48, 43/50) Lymph node (bronchial): histiocytic hyperplasia (0/38, 25/37, 39/43) Nose: cytoplasmic alteration (29/46, 37/46, 40/50)
Level of evidence of carcinogenic activity	Some evidence	Clear evidence	No evidence	No evidence

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EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (*clear evidence* and *some evidence*); one category for uncertain findings (*equivocal evidence*); one category for no observable effects (*no evidence*); and one category for experiments that cannot be evaluated because of major flaws (*inadequate study*). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

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NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on talc on June 23-24, 1992, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

NOTE: A summary of the Technical Reports Review Subcommittee's remarks will appear in a future draft of this report.

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INTRODUCTION

TALC (Non-Asbestiform)

CAS No. 14807-96-6

Molecular Formula: $\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$ Molecular Weight: 379.26

Synonyms: Talcum; Agalite; Emtal 596; non-asbestiform talc; non-fibrous talc; Steatite; hydrous magnesium silicate

CHEMICAL AND PHYSICAL PROPERTIES

Talc is a fine powder, white to grayish white in color, with a greasy feel and luster. It is insoluble in water, cold acids, and alkalis (*Merck Index*, 1983), has a density of 2.7 to 2.8, and a melting point of 900° to 1,000° C (Hawley, 1977). Talc as a pure mineral is composed of 63.5% SiO_2 , 31.7% MgO , and 4.8% H_2O (Pooley and Rowlands, 1977).

PRODUCTION, USE, AND HUMAN EXPOSURE

Talc is produced by open pit or underground mining of talc rocks and processed by crushing, drying, and milling. Contaminating minerals including iron, nickel, manganese, chromium, aluminum, and titanium are separated from talc by flotation or elutriation. Talc is then finely powdered, treated with boiling diluted hydrochloric acid, washed well, and dried (Osol, 1980). Geological formation of talc rock results from the alteration of magnesia- and silica-rich ultramafic rocks under a range of temperatures and pressures. These hydrothermal alterations may lead to the formation of other mineral phases such as tremolite and serpentine minerals, including chrysotile. These mineral phases may occur as microscopic intergrowths, nodules, or discrete zones within or adjacent to talc (Rohl *et al.*, 1976).

United States production of talc for 1985 was estimated at 1.1 million metric tons, with industrial pattern of use as follows: ceramics, 37%; paints, 19%; paper, 10%; roofing, 10%; plastics, 7%, cosmetics, 5%; rubber, 3%; insecticides, 1%; and other uses, 9% (Bureau of Mines, 1986). Commercial talc is categorized into cosmetic grade, which is free of asbestos, and industrial grade, which contains

other minerals including asbestos (Hildick-Smith, 1976).

A comprehensive review of the literature before 1987 on the use, exposure, and biological effects of talc was published by IARC (1987). Talc is used as a dusting powder, including baby powder, either alone or with starch or boric acid, for medicinal or toiletry preparations; as an excipient and filler for pills and tablets; and for dusting tablet molds (*Merck Index*, 1983). It is also used as a filler and pigment for paints, putty, and plaster; as a carrier and diluent for pesticides; as an additive to clay in ceramic manufacture; in paper coatings; and for the manufacture of rubber and roofing materials (Hawley, 1977). The recommended time-weighted average (TWA) human exposure level for talc containing no asbestos fibers is 2 mg/m^3 (ACGIH, 1989).

A large segment of the population is potentially exposed to talc. The number of workers exposed to talc was estimated at 1,371,201, which includes 349,228 females (NIOSH, 1990). In addition, the public is potentially exposed to talc through its many uses in pharmaceuticals and consumer products. Based on its uses, human exposure to talc can occur via inhalation, ingestion, or dermal exposure.

ABSORPTION, DISTRIBUTION, AND EXCRETION

Experimental Animals

The absorption and disposition of ^3H -labeled talc in rats, mice, and guinea pigs administered a single oral dose, as well as its translocation in rabbits administered a single or multiple intravaginal dose was studied by Phillips *et al.* (1978). The oral doses

were 50 mg/kg for rats, 40 mg/kg for mice, and 25 mg/kg for guinea pigs. Rabbits were given either a single intravaginal dose of 50 mg/kg or the same dose once a day for 6 days. In rats, mice, and guinea pigs, more than 95% of the dose was excreted in the feces 3 to 4 days after dosing. Less than 2% of the radioactivity was recovered in the urine. This radioactivity probably reflected contamination of urine samples with feces. No radioactivity was found in the liver or kidneys of these animals. No translocation of talc was found in the ovaries of rabbits.

Hanson *et al.* (1985) and Pickrell *et al.* (1989) studied the lung burden in groups of 5 male and 5 female F344/N rats and B6C3F₁ mice following inhalation exposure to concentrations of talc for 6 hours daily, 5 days per week, for 4 weeks. The mean exposure concentrations used were 2.3, 4.3, or 17 mg/m³ for rats and 2.2, 5.7, or 20.6 mg/m³ for mice. The resulting lung talc burdens were 0.08, 0.19, and 0.87 mg/g of lung for rats and 0.1, 0.33, and 1.2 mg/g of lung for mice. These data clearly indicate that the amount of talc retained per unit of lung tissue was proportional to the exposure concentration of talc.

Pulmonary deposition, translocation, and clearance of neutron-activated talc was studied in hamsters after a single, 2-hour, nose-only inhalation exposure (Wehner *et al.*, 1977a,b). Deposition of talc in the lung was demonstrated by X-ray fluorescence and X-ray diffraction. An estimated 6% to 8% of the inhaled quantity was deposited in the alveoli. The biological half-life of the talc deposited in the alveoli was estimated at 7 to 10 days. No translocation of talc to liver, kidneys, ovaries, or other parts of the body was found.

Humans

Talc, a filler in some drugs injected by addicts, was found in the lung (Groth *et al.*, 1972; Lamb and Roberts, 1972; Farber *et al.*, 1981; Crouch and Churg, 1983), spleen, kidney, liver, brain, adrenal gland, thyroid gland (Groth *et al.*, 1972), and retina (Atlee, 1972) of some addicts. In the lung, most of the talc particles were seen within the vessels of the alveolar walls and were often associated with marked foreign body granulomas (Crouch and Churg, 1983).

TOXICITY

Experimental Animals

The LD₅₀ for talc has not been established. Talc caused death in guinea pigs given 2 or 3 injections of 25 mg talc in saline (Dogra *et al.*, 1977) and in rats receiving a splenic injection of 1,400 mg/kg body weight (Eger and DaCanalis, 1964). Deaths occurred in rats exposed to a very dense atmosphere of talc (particle size <5 µm) 3 hours a day, for 12 days (Policard, 1940). The concentration of talc in the atmosphere was not known and the observed mortality may have been due to suffocation.

Wagner *et al.* (1977) reported on the toxic effects of talc in rats exposed orally or by inhalation. No significant decrease in mean life span and no pathologic effects were found in rats fed 100 mg talc for 101 days. Rats exposed to talc atmospheres of 10.8 mg/m³ (particle size, 25 µm) for 3 months showed minimal lung fibrosis, and no change in severity occurred during the postexposure period. By contrast, rats exposed to the same atmospheres for 1 year showed minimal to slight fibrosis, and the severity had increased to moderate within a year after cessation of exposure. Rats exposed to atmospheres of 30 to 383 mg/m³ "industrial" or "pharmaceutical" talc for 9 months developed chronic inflammatory changes, including thickening of the pulmonary artery walls and emphysema (Bethege-Iwanska, 1971). Hamsters exposed to respirable aerosols containing 8 mg/m³ of cosmetic grade talc for 150 minutes a day, 5 days per week, for 300 days showed no histopathologic changes in the lung, heart, liver, renal tissue, or uterus (Wehner, 1980).

Rats given a single intratracheal injection of 50 mg of pure talc in water did not show lung fibrosis or lymph node abnormalities. Those given the same dose of "calcined" talc developed lung and lymph node fibrosis (Luchtrath and Schmidt, 1959). These differing results may be related to differences in the crystal structures of "pure" and "calcined" talc. Bronchiolar inflammation occurred in rats 4 days after an intratracheal injection of 25 mg of talc (containing tremolite) in water; collagenous tissue developed within a few months after injection (Gross *et al.*, 1970).

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Injection of 10 mg of talc containing some asbestos into the pleural cavity of mice produced granulomas (Davis, 1972). A single injection of 20 mg of talc into the right pleural cavity of rats produced granulomas at the injection site; one lung adenoma was also observed but no other changes related to talc administration were observed in the lung (Wagner *et al.*, 1977). Rats with abdominal muscle implants of suture materials dusted with talc or talc pellets initially showed mild to moderate acute inflammation, followed by chronic inflammation and granuloma formation within 3 days (Sheikh *et al.*, 1984).

Rats with subcutaneous inflammation caused by talc had a decrease in bone formation as evidenced by hypozincemia and a decrease in metaphyseal trabecular surfaces. Both hypozincemia and the decrease in osteoblast trabecular surfaces were directly proportional to the number of granulomas present (Marusic *et al.*, 1990).

Talc produced retinopathy in adult Rhesus monkeys given intravenous injections of talc once every 2 weeks for 3.5 to 10 months. Talc particles were found lodged in the precapillary arterioles and capillaries, producing a focal occlusion of retinal and choroidal capillaries (Kaga *et al.*, 1982a,b).

Humans

Exposure to industrial grade talc dust causes pulmonary fibrosis, however, reports on cosmetic grade talc dust are conflicting. Hildick-Smith (1976) reported that cosmetic grade talc did not appear to be injurious to health, while Vallyathan and Craighead (1981) reported that it was. Four of seven workers exposed to heavy concentrations (0.4 to 36 mg/m³) of cosmetic grade talc for 4 to 27 years had histologic evidence of pulmonary fibrosis at death (Thariault *et al.*, 1974). Wells *et al.* (1979) also noted chronic pulmonary degenerative disease in a housewife who reported heavy use of cosmetic talc. Inhalation of pure talc is known to result in a disease known as talcosis, which may include acute or chronic bronchitis and interstitial inflammation. Radiographically, the lesion appears as a small, irregular nodule, typical of a small-airway obstruction. Intravenous administration of talc-containing oral medications by abusers causes vascular granulomas (Feigin, 1986). Intravenous talcosis was diagnosed in a 36-year-old woman who was a drug abuser (Hill *et al.*, 1990). Talcosis in this patient was identified by the presence of peripheral nodular lesions on chest X-rays and was confirmed by the presence of birefringent particles

in a transbronchial biopsy. Pulmonary talc granulomatosis was diagnosed in a cocaine sniffer (Oubeid *et al.*, 1990). Chest X-rays of a heroin addict who later died of respiratory failure showed a progressive massive fibrosis of the lung secondary to intravenous injection of the drug (Crouch and Churg, 1983). Microscopic examination of lung lesions revealed an active granulomatous reaction with associated vascular obliteration. Throughout the lesion, refractile birefringent plates of particulate material were noted. Interstitial perivascular and vascular granulomas were noted in the periphery of the lung. The particulate material was identified as talc by X-ray spectroscopy and diffraction methods. Intravenous injection of talc-containing drugs intended for oral use was the cause of pulmonary granulomatosis and pulmonary hypertension in 19 patients (Arnett *et al.*, 1976). In patients with pulmonary hypertension, talc granuloma was found in the pulmonary arteries. In patients without hypertension, talc granuloma was found in the pulmonary interstitium. Patients suffering from talc granulomatosis (confirmed by lung biopsy) as a result of intravenous injection of crushed tablets of pentazocine, had dyspnea, increased angiotensin-converting enzyme concentrations, and increased lymphocytes by bronchoalveolar lavage (Farber *et al.*, 1982). Pneumoconiosis (talcosilicosis) was diagnosed in a 54-year-old female confectionery worker who was exposed to talc dust for 5 years (Canessa *et al.*, 1990). Talc, given by intrapleural instillation to promote pleural symphysis in the palliation of recurrent malignant pleural effusions, caused adult respiratory distress syndrome (ARDS) in three patients (Rinaldo *et al.*, 1983). Symptoms of ARDS included fever, dyspnea, and respiratory failure. ARDS occurred in a 16-month-old baby inhaling baby powder. Normal pulmonary function returned in this patient after 6 years, as determined by a follow-up study (Reyes and Brown, 1989).

CARCINOGENICITY

Experimental Animals

Results of carcinogenicity studies of talc in animals were reviewed by the IARC (1987). The following is an excerpt of this review:

No significant difference in tumor incidence was observed between two groups of Wistar rats (25 animals/sex/group, 10 weeks old) given an equivalent of 50 mg/kg per day of commercial talc (composition not specified) in the diet or the basal diet for life (Gibel *et al.*, 1976). Similar results were obtained in groups of 16 male and 16 female

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Wistar rats (21 to 26 weeks old) given 100 mg of Italian talc (particle size, 25 mm; containing 92% talc, 3% chlorite, 1% carbonate minerals, and 0.5 to 1% quartz) per rat per day in the diet or the basal diet for 5 months and observed for life (Wagner *et al.*, 1977). In both studies small numbers of animals were used.

Groups of 24 male and female Wistar rats, 6 to 8 weeks of age, were exposed by inhalation to 10.8 mg/m³ Italian talc aerosol 7.5 hours a day, 5 days per week, for 6 or 12 months. Ten days after the end of each exposure period, 6 rats in each group were killed; an additional 4 rats were killed one year later. Within 28 months from the beginning of the study, 12 animals in each group had died. No lung tumors were observed in rats exposed to talc for 6 months; one lung adenoma occurred among rats exposed for 12 months. No lung tumors were found in the control rats (Wagner *et al.*, 1977). The adequacy of this study is in question because only a small number of rats survived longer than 12 months.

Three groups of 50 male and female hamsters, 4 weeks of age were exposed to talc aerosol (37.1 mg/m³, mean respirable fraction 9.8 mg/m³) for 3, 30, or 150 minutes per day, 5 days a week, for 30 days. Two additional groups of hamsters were exposed to talc aerosol (27.4 mg/m³, mean respirable fraction 8.11 mg/m³) for 30 or 150 minutes per day, for 300 days. Two groups of 25 male and female hamsters were exposed to air and served as controls. No primary tumors were found in the respiratory system of any hamster. Twenty-five percent of the hamsters exposed to the aerosols for 30 or 150 minutes for 300 days had alveolar cell hyperplasia compared to 10% in the controls (Wehner *et al.*, 1977a, 1979). The exposure duration of this study was short and considered inadequate.

No local tumors were found in 50 female R3 mice, 3 to 6 months of age, given a 0.2 mL subcutaneous injection of talc of unspecified composition (80 mg talc in peanut oil) and observed for life (Neukomm and de Trey, 1961).

Forty Swiss albino rats, 6 weeks of age and sex unspecified, received a single intraperitoneal injection of 20 mg commercial talc (unspecified composition) in saline. Sixteen animals died by the end of 6 months. Of the 24 mice that lived to termination (time not specified) three had peritoneal mesotheliomas compared to 3 of 46 of the controls

(Ozesmi *et al.*, 1985). This study was considered inadequate because of poor reporting.

Forty female Wistar rats, 8 to 12 weeks of age, were given four intraperitoneal injections of 25 mg granular talc in 2 mL saline at weekly intervals. Similarly, 80 females were injected with saline and served as controls. The rats were observed until termination or death (average survival time, 602 days). A mesothelioma occurred in 1 of 36 rats given talc but none was found in the controls (Pott *et al.*, 1974, 1976a,b).

No mesothelioma was observed in two groups of 24 male and female Wistar rats given a single intrapleural injection of 20 mg Italian talc in saline or saline alone. A pulmonary adenoma occurred in one rat that died at 25 months. Mean survival time (655 days for the talc group versus 691 for the controls) was not affected (Wagner *et al.*, 1977).

Groups of 30 to 50 female Osborne-Mendel rats, 12 to 20 weeks of age, received intrapleural implantation of one of seven grades of refined commercial talc from separate sources in hardened gelatin. Rats were observed for up to 2 years at which time survivors were killed. Pleural sarcoma incidences were: grade 1, 1/26; grade 2, 1/30; grade 3, 1/29; grade 4, 1/29; grade 5, 0/30; grade 6, 0/30; grade 7, 0/29. The incidence of pleural sarcoma was 3 of 491 in untreated controls, 17 of 615 in controls receiving implants of "nonfibrous" material described by the authors as "noncarcinogenic," and 14 of 29 in rats receiving UICC crocidolite asbestos (Stanton *et al.*, 1981).

The IARC Working Group noted that in most of the talc studies, no or limited characterization of the mineralogy, fiber content, or particle size of the samples was given. Thus, the group concluded that there was inadequate evidence on the carcinogenicity of talc to experimental animals.

Humans

An epidemiological study of pottery workers in the United States revealed an association between exposure to non-fibrous talc and increased mortality and lung cancer incidence (Thomas and Stewart, 1987). Increased incidences of lung cancer occurred exclusively among pottery workers employed in the manufacture of plumbing fixtures. A later study of employees in three ceramic plumbing fixture factories showed increased mortality from benign respiratory disease and from lung cancer. The

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increased incidence in lung cancer was highest among workers who were simultaneously exposed to silica and talc. The lung cancer mortality risk increased with the number of years of exposure to talc, but showed no pattern by the number of years of exposure to silica. Among men exposed to talc, lung cancer risk decreased with age at first exposure to non-fibrous talc and increased with years since first exposure (Thomas, 1990). Whether or not exposure to silica had a promoting effect on lung cancer is not known. No increased risk for lung cancer or benign respiratory disease was found among miners of non-asbestiform talc or talc millers (Wergeland *et al.*, 1990).

A case-control study showed that women who had perineal exposure to deodorizing powders alone or in combination with other talc-containing powders, had a 2.8 times higher risk of developing borderline ovarian tumors than women who were not perineally exposed to powder (Harlow and Weiss, 1989). In an earlier study, the use of talc as a dusting powder on the perineum or on sanitary napkins by women was associated with an increased risk of epithelial ovarian cancer. Women engaged in both practices had a relatively higher risk of developing this type of cancer (Cramer *et al.*, 1982). No information was presented regarding exposure levels or the content of contaminating minerals of the talc used. In another study, the role of exposure to talcum powder, tobacco, alcohol, and coffee, and the histories of tubal sterilization and hysterectomy on ovarian cancer risk was assessed. The study involved 188 women diagnosed with epithelial ovarian cancer and 539 control women. No association was found between the incidence of epithelial ovarian cancer and increasing frequency or duration of talc use, and patients did not differ from control women in the use of talc on sanitary pads, contraceptive diaphragms, or both. (Whittemore *et al.*, 1988).

REPRODUCTIVE AND TERATOGENIC EFFECTS

Experimental Animals

Talc produced nonspecific abnormalities in chicken eggs at incidences similar to those caused by thalidomide and sulphadimethoxine (Yang, 1977).

No teratologic effects were observed in hamsters, rats, mice, or rabbits after oral administration of talc. The doses used were 1,600 mg/kg for rats and mice on days 6 through 15 of gestation, 1,200 mg/kg for hamsters on days 6 through 10 of gestation, and 900 mg/kg for rabbits on days 6 through 18 of gestation (Food and Drug Research Laboratories, 1973).

Humans

No information on the reproductive or teratogenic effects of talc in humans has been reported.

GENETIC TOXICOLOGY

There are no published studies on the genotoxicity of talc. The IARC (1987) review of talc included unpublished results from a 1974 study conducted by Litton Bionetics that showed no mutagenic activity for talc *in vitro* or *in vivo*. Talc did not induce mutations in *Salmonella typhimurium* strains TA1530 or HisG46, or in the yeast, *Saccharomyces cerevisiae*. No chromosomal aberrations were observed in human fibroblasts treated with talc *in vitro*. *In vivo* tests conducted in rats gave negative results for induction of chromosomal aberrations in bone marrow cells and dominant lethal mutations in germinal cells.

STUDY RATIONALE

Talc was nominated by NIOSH in 1978 for testing by NTP because of the paucity of adequate information on its carcinogenicity and because of widespread human exposure. The inhalation route was chosen because it is the most common route for human exposure.

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MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF TALC

Talc (MP 10-52 Grade) was obtained from Walsh and Associates (North Kansas City, MO) in two lots (lot numbers W101882 and B5415). The talc purchased was manufactured by the Minerals, Pigments, and Metals Division of Pfizer, Inc. and is one of their microtalc series of products. Both lots were from Pfizer's Barretts, Montana, mine which is a strip mine located between Barretts and Three Brother, Montana. This mine is the only source for the MP 10-52 grade talc. The grade designation is for high purity talc that has a top particle size of 10 μm and according to the manufacturer contains no tremolite or any asbestiform minerals. Lot W101882 was used from the beginning of the 2-year studies through January 1986. Lot B5415 was used in the 2-year studies from 27 January 1986 to the end of the studies on 31 October 1986. The talc was extensively characterized by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and by McCrone Associates (Norcross, GA). The methods and results of these studies are detailed in Appendix H.

The study mineral, a finely powdered white solid, was identified as talc by infrared spectroscopy, elemental analysis, Karl Fischer water analysis, thermogravimetric analyses, spark source mass spectrometry, automated scanning electron probe analyses, X-ray diffraction, polarized light microscopy, and transmission electron microscopy. Both lots were shown to be asbestos free by polarized light microscopy and transmission electron microscopy. Results of automated scanning electron microprobe analysis of lot W101882 indicated that the sample was virtually free of silica (1 particle of silica in 1,466 particles examined). Bulk chemical stability studies were not conducted due to the physical and chemical properties of talc. During the study the compound was stored in tightly sealed plastic bags at 25° C.

GENERATION AND MONITORING OF CHAMBER CONCENTRATIONS

Talc aerosols were generated in a single fluidized-bed generator (FBG) by injecting compressed air

into the bed (Figure H2). The aerosolized talc particles were then mixed with diluting air before being delivered to the exposure chambers (Hazelton 1000 and 2000, Lab Products, Inc.). A second FBG for the control chamber contained only the stainless steel bed material (Figures H3 and H4).

Aerosol concentrations were monitored each day in each chamber by taking three, 2-hour filter samples. Background concentrations of suspended particles were measured each day in the control chamber by taking a 6-hour filter sample. A RAM-S forward light scattering monitor (GCA, Bedford, MA) was used to determine the stability of the aerosol concentrations and the need to adjust the aerosol generation system during the exposure. Determinations were made at the beginning, middle, and end of each filter sampling period. The overall mean concentrations were 5.9 and 16.7 mg/m^3 for the mouse study and 6.1 and 18.6 mg/m^3 for the rat study. While the overall means were very close to target concentrations, there were problems experienced in maintaining control of chamber concentrations. Weekly mean exposure concentrations for the 2-year studies are presented in Figures H5 through H8.

Chamber Atmosphere Characterization

Uniformity of the aerosol concentrations in each chamber was determined at approximately 3-month intervals with the RAM-S. The spatial variation as estimated by the relative standard deviation (RSD) was higher in the mouse study than the rat study with values ranging from 12% to 44% (RSD) for the mice and 2% to 31% (RSD) for the rats. To minimize the variation in talc concentrations, the animal cages were rotated once each week.

The time to reach 90% of the target concentration (T_{90}) was approximately 10 minutes. Therefore, the length of the exposure was defined at 6 hours plus the T_{90} of 10 minutes.

The aerosol size distribution was determined once each month for each chamber using a cascade impactor. The average mass mean aerodynamic diameter (MMAD) and the geometric standard deviation (σ_g) were calculated to be $3.3 \pm 1.9 \mu\text{m}$ and $3.6 \pm 2.0 \mu\text{m}$ for the 6 and 18 mg/m^3 mouse

chambers. The values were $2.7 \pm 1.9 \mu\text{m}$ and $3.2 \pm 1.9 \mu\text{m}$ for the 6 and 18 mg/m^3 rat chambers. The individual values are presented in Tables H1 and H2.

Study Design

Groups of 50 male and 50 female rats and mice were selected for whole body inhalation to talc at target concentrations of 0 (chamber controls), 6, or 18 mg/m^3 . Rats were exposed for 6 hours daily, 5 days a week until mortality in any exposure group reached 80% (113 weeks for males and 122 weeks for females). Exposure of rats to talc was extended beyond 2 years based on the report that 80% of pulmonary neoplasms induced in rats by inhalation exposure to diesel exhaust occurred after 2 years (Mauderly *et al.*, 1986). Mice were exposed for 103 or 104 weeks. At the conclusion of the exposures, rats were exposed to filtered air for 10 or 11 days, while mice were exposed to filtered air for 10 to 14 days. All animals were subjected to necropsy and a complete pathology evaluation.

Additional special study groups of 22 male and 22 female rats and 40 male and 40 female mice similarly exposed to 0, 6, or 18 mg/m^3 were designated for interim pathology evaluations; lung talc burden measurements; serial pulmonary function measurements (rats only); and lung biochemistry, cytology, and phagocytosis measurements. Rats were evaluated at 6, 11, 18, and 24 months, while mice were evaluated at 6, 12, and 18 months. Insufficient numbers of rats remained alive at week 103 of exposure for both pulmonary function and/or lung biochemistry/cytology and pathology distribution groups, therefore the remaining rats in these groups were combined. The numbers of rats and mice evaluated for pulmonary function and lung biochemistry, cytology, and phagocytosis and the methods used for each of the parameters are presented in Appendix F for rats and Appendix G for mice.

Source and Specification of Animals

Male and female F344/N rats were obtained from Lovelace Inhalation Toxicology Research Institute (Albuquerque, NM). Male and female B6C3F₁ mice were obtained from Frederick Cancer Research Center (Frederick, MD). Rats and mice were held 3 weeks before the studies began. Rats were 6 to 7 weeks old, and mice were 7 weeks old, when the studies began. Animal health was monitored by serologic analyses during the studies under the protocols of the NTP Sentinel Animal Program.

Animal Maintenance

Rats and mice were housed individually throughout the studies. Drinking water was available *ad libitum*. Further details of animal maintenance are given in Table 1.

Clinical Examinations and Pathology

All rats and mice were observed twice daily. Clinical observations and body weights were recorded at the beginning of the studies, weekly for 13 weeks, and monthly thereafter.

A necropsy was performed on all rats in the lifetime core study and all mice in the 2-year core study. Organ weights were recorded for the brain, heart, right kidney, liver, and lungs at the end of the studies. During necropsy, all organs and tissues were examined for grossly visible lesions. A complete histopathologic examination was performed on all animals. Tissues for microscopic examination were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned to a thickness of 5 μm , and stained with hematoxylin and eosin.

Microscopic evaluations were completed by the study laboratory pathologist and the pathology data were entered into the Toxicology Data Management System (TDMS). The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit for accuracy of labeling and animal identification and for thoroughness of tissue trimming. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. A quality assessment pathologist reviewed lung and bronchial and mediastinal lymph nodes in rats and mice and nose in male mice for accuracy and consistency of lesion diagnosis.

The quality assessment report and slides were submitted to the Pathology Working Group (PWG) chair, who reviewed tissues for which there was a disagreement in diagnosis between the laboratory and quality assessment pathologists. All pulmonary neoplasms in female rats and representative histopathology slides of adrenal gland (rats), bronchial lymph node, lung, mediastinal lymph node (rats), and nose, or lesions of general interest were presented by the chair to the PWG for review. The PWG included the quality assessment pathologist as

well as other pathologists experienced in rodent toxicologic pathology who examined these tissues without knowledge of dose group or previously rendered diagnoses. When the consensus diagnosis of the PWG differed from that of the laboratory pathologist, the final diagnosis was changed to reflect the opinion of the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analysis of pathology data, the diagnosed lesions for each tissue type were evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the Results section of this report. Animals were censored from the survival analyses at the time they were found dead from other than natural causes or were found to be missing; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table tests to identify dose-related trends. All reported P values for the survival analysis are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions presented in Tables A1, A4, B1, B4, C1, C4, D1, and D4 are given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals with that site examined microscopically. For calculation of statistical significance, the incidences of all nonneoplastic lesions and most neoplasms (Tables A2, B2, C2, and D2) are also given as the ratio of the number of affected animals to the number of animals with the site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., skin, intestine, hardenian gland, and mammary gland) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Neoplasm Incidences

The majority of tumors in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was logistic regression analysis, which assumed

that the diagnosed tumors were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, tumor prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not significantly enhance the fit of the model. The dosed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When tumors are incidental, this comparison of the time-specific tumor prevalences also provides a comparison of the time-specific tumor incidences (McKnight and Crowley, 1984).

In addition to logistic regression, alternative methods of statistical analysis were used, and the results of these tests are summarized in the appendices. These include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal tumors, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of tumor-bearing animals.

Tests of significance include pairwise comparisons of each dosed group with controls and a test for an overall dose-response trend. Continuity-corrected tests were used in the analysis of tumor incidence, and reported P values are one sided. The procedures described above also were used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, see Haseman (1984).

Analysis on Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in this study were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which lesion prevalence was modeled as a logistic function of chemical exposure and time. For lesions detected at the interim evaluation, the Fisher exact test was used, a procedure based on the overall proportion of affected animals.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data that had

approximately normal distributions were analyzed using the parametric multiple comparison procedures of Williams (1971, 1972) and Dunnett (1955). Lung burden parameters that had skewed distributions were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-response trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-response trend (Dunnett's or Dunn's test).

Quality Assurance Methods

The lifetime and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as study records were submitted to the NTP Archives, they were audited by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and preliminary review draft of this NTP Technical Report were conducted. Audit procedures are presented in the reports, which are on file at the NIEHS. The audit findings were reviewed and assessed by the NTP staff so that all discrepancies had been resolved or were otherwise addressed during the preparation of this Technical Report.

Materials and Methods

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TABLE 1
Experimental Design and Materials and Methods in the Lifetime and 2-Year Inhalation Studies of Talc

Study Laboratory Lovelace Inhalation Toxicology Research Institute (Albuquerque, NM)
Strain and Species Rats: F344/N Mice: B6C3F ₁
Animal Source Rats: Lovelace Inhalation Toxicology Research Institute (Albuquerque, NM) Mice: Frederick Cancer Research Center (Frederick, MD)
Time Held Before Studies 3 weeks
Average Age When Placed on Studies 6-7 weeks
Date of First Exposure Rats: 2 July 1984 Mice: 4 June 1984
Duration of Exposure Rats: 6 hours/day, 5 days/week for 113 weeks (males) and 122 weeks (females) Mice: 6 hours/day, 5 days/week for 103-104 weeks
Date of Last Exposure Rats: 29 August 1986 (males) and 31 October 1986 (females) Mice: 30 May 1986
Average Age When Killed Rats: 120-121 weeks (males) and 129-130 weeks (females) Mice: 110-112 weeks
Method of Sacrifice Injection of T-61 solution for all rats in the lifetime study, all rats designated for pathologic evaluation, and all mice. Halothane anesthesia for all rats designated for biochemical interim evaluations.
Necropsy Dates Rats: 8-9 September 1986 (males) and 10-11 November 1986 (females) Mice: 9-13 June 1986 (males) and 2-6 June 1986 (females)
Size of Study Groups 50 males and 50 females
Method of Animal Distribution Assigned to groups by weight and sex using computer-generated random numbers.
Animals per Cage 1
Method of Animal Identification Toe clip and ear tag
Diet NIH-07 Rat and Mouse Ration (Zeigler Bros., Gardner, PA) available <i>ad libitum</i> during nonexposure periods
Maximum Storage Time for Feed 90 days

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TABLE 1
Experimental Design and Materials and Methods in the Lifetime and 2-Year Inhalation Studies of Talc
(continued)

Water

Automatic Watering System (Edstrom), available *ad libitum*

Cages

Stainless steel mesh cages (Hazleton, Aberdeen, MD)

Chambers

Rats: Stainless steel multitiered whole-body exposure chambers (H2000, Hazleton Systems, Aberdeen, MD), washed once weekly

Mice: Stainless steel multitiered whole-body exposure chambers (H1000, Hazleton Systems, Aberdeen, MD), washed once weekly

Bedding

Untreated paper cage board (Shepherd Specialties Paper, Inc., Kalamazoo, MI), changed twice a day

Filters

Room Air and Chamber Air High Efficiency Particulate Air (HEPA) Filter (prefilter and exit filter), MIL Spec MIL-F-51068C (Flanders, Washington, DC)

Animal Room Environment

Rats

Average temperature: 24° C

Relative humidity: 9%-100%

Fluorescent light: 12 hours/day

Room air changes: minimum of 10 changes/hour

Mice

Average temperature: 24° C

Relative humidity: 10%-100%

Fluorescent light: 12 hours/day

Room air changes: minimum of 10 changes/hour

Exposure Concentrations

0, 6, and 18 mg/m³ by inhalation

Type and Frequency of Observation

Observed twice daily; body weights and clinical findings recorded at study initiation, weekly through week 13, and monthly thereafter

Necropsy

Necropsy performed on all animals. Organ weights recorded for brain, heart, right kidney, liver, and lung.

Histopathology

Complete histopathologic examinations performed on all animals. In addition to tissue masses and gross lesions, tissues examined included: adrenal gland, bone (including marrow), brain, clitoral gland (female rats), epididymis, esophagus, gallbladder (mice), harderian gland (female rats and mice), heart, kidney, large intestine (cecum, colon, rectum), larynx, liver, lung, lymph nodes (bronchial, mandibular, mediastinal, mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland (male rats), prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, ileum, jejunum), spleen, stomach (forestomach, glandular), testis, thymus, thyroid gland, trachea, urinary bladder, and uterus.

RESULTS

RATS

4-WEEK STUDY DOSE SELECTION

Selection of 6 or 18 mg talc/m³ as the exposure concentrations was based on the results of a 4-week inhalation study in F344/N rats to determine lung talc burden and histopathologic changes associated with talc exposure. These studies indicated that the amount of talc retained in the lung was similar between sexes and proportional to exposure concentration (Appendix K). Microscopic examination of the lungs revealed an accumulation of alveolar macrophages in the lungs only at the 18 mg/m³ concentration. Based on these findings it was predicted that aerosol concentrations greater than 18 mg/m³ would overwhelm lung clearance mechanisms, impair lung function, and possibly shorten survival.

LIFETIME STUDY

Survival

Estimates of survival probabilities for male and female rats are shown in Table 2 and in the Kaplan-Meier curves in Figure 1. Survival of exposed male and female rats was similar to that of the controls.

Body Weights and Clinical Findings

The mean body weights of male and female rats exposed to 6 mg/m³ talc were similar to those of controls throughout the study (Tables 3 and 4, and Figure 2). Mean body weights of male and female rats exposed to 18 mg/m³ were slightly lower than those of controls, particularly after week 65. The final mean body weight of males in the 18 mg/m³

group was 4% lower than that of the controls, while the final mean body weight of females in the 18 mg/m³ group was 14% lower than that of the controls.

Serological tests were performed prior to the beginning of the study and after 6, 12, and 18 months of exposure; serological tests were negative for all microorganisms tested (Table J1). After 24 months and 28 and 30 months (females), the serological tests were positive for Kilham rat virus (KRV), Sendai virus, and rat coronavirus/sialodacryoadenitis virus (RCV/SDA). The significance of the positive KRV titer is unknown since it was found in only one rat and was not observed at later times. No clinical findings or gross or microscopic lesions that could be attributed to Sendai virus or RCV/SDA infections were observed in the talc exposed or control groups. Since there was no clinical or pathological evidence of disease and since the infection occurred very late in the study, these subclinical infections are believed to have had no impact on the study results.

Pathology and Statistical Analyses of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplastic or nonneoplastic lesions of the lung, lymph node, nose, and adrenal medulla. Summaries of the incidences of nonneoplastic lesions and neoplasms, the individual animal neoplasm diagnoses, and the statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one group are presented in Appendix A for male rats and Appendix B for female rats.

TABLE 2
Survival of Rats in the Lifetime Inhalation Study of Talc

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Lifetime Study Groups			
Animals initially in study	50	50	50
Natural deaths	18	17	14
Moribund kills	23	19	20
Animals surviving to study termination	9	14	16
Percent survival at end of study ^a	18	28	32
Mean survival (days) ^b	696	707	711
Survival analysis ^c	P=0.217N	P=0.422N	P=0.192N
Special Study Groups^d			
Animals initially in study	22	22	22
Natural deaths	2	2	6
Moribund kills	9	5	6
Scheduled sacrifice	11	15	10
Females			
Lifetime Study Groups			
Animals initially in study	50	50	50
Natural deaths	11	19	14
Moribund kills	28	17	27
Missing ^d	0	1	0
Animals surviving to study termination	11	13	9
Percent survival at end of study ^a	22	28	18
Mean survival (days) ^b	743	753	758
Survival analysis ^c	P=0.846	P=0.805N	P=0.977
Special Study Groups^d			
Animals initially in study	22	22	22
Natural deaths	2	1	2
Moribund kills	5	3	8
Scheduled sacrifice	15	18	12

^a Kaplan-Meier determinations

^b Mean of all deaths (uncensored, censored, and terminal sacrifice).

^c The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns. A negative trend or lower mortality in a dose group is indicated by N.

^d Censored from survival analyses

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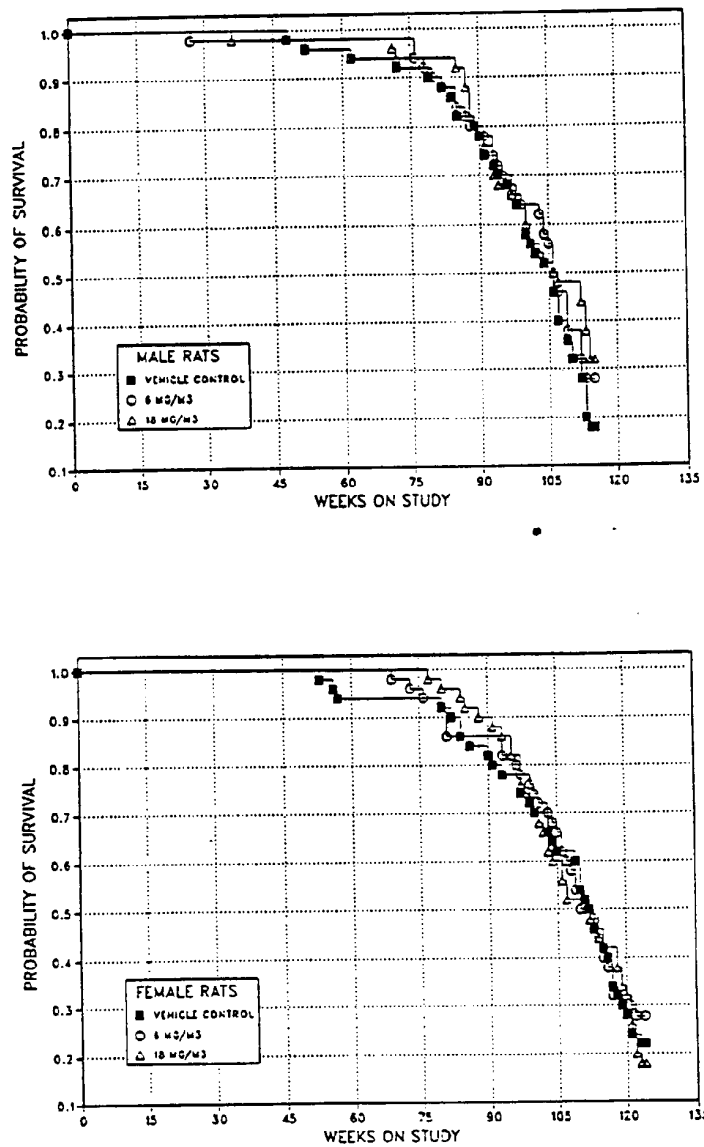


FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Rats Administered Talc by Inhalation
for Their Lifetime

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TABLE 3
Mean Body Weights and Survival of Male Rats in the Lifetime Inhalation Study of Talc

Weeks on Study	0 mg/m ³		6 mg/m ³			18 mg/m ³		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	118	72	121	103	72	119	101	72
2	174	72	174	100	72	174	100	72
3	201	72	200	100	72	202	101	72
4	225	72	215	95	72	219	97	72
5	237	72	239	101	72	238	101	72
6	250	72	252	101	72	251	100	72
7	265	72	263	99	72	263	99	72
8	275	72	270	98	72	269	98	72
9	287	72	280	98	72	281	98	72
10	297	72	293	99	72	293	99	72
11	304	72	300	99	72	297	98	72
13	317	72	315	100	72	312	98	72
17	339	72	338	100	72	331	98	72
21	359	72	355	99	72	351	98	72
25	374	71	370	99	72	367	98	72
29 ^a	380	68	378	99	68	369	97	69
33	398	68	393	99	68	386	97	69
38	407	68	405	100	68	393	97	68
41	413	68	412	100	68	401	97	68
45	421	68	420	100	68	410	97	68
49 ^a	431	63	428	99	65	418	97	65
53	434	62	432	100	65	422	97	65
57	435	62	432	99	65	424	97	65
61	443	62	442	100	65	430	97	65
65	450	61	444	99	65	432	96	65
69	448	61	440	98	65	429	96	65
73	453	60	442	98	65	432	95	63
77	452	60	441	98	63	429	95	62
81 ^a	444	55	434	98	57	423	95	59
85	450	49	434	97	53	424	94	57
89	447	47	437	98	50	424	95	51
93	434	43	429	99	48	408	94	46
97	429	40	427	100	41	407	95	40
101	410	34	395	96	40	394	96	34
105 ^a	390	29	391	100	35	385	99	28
109	377	18	390	104	19	376	100	24
113	358	11	389	109	15	342	96	21
Terminal sacrifice		9			14			16
Mean for weeks								
1-13	246		244	99		243	99	
14-52	391		389	99		381	97	
53-113	428		425	99		411	96	

^a Interim evaluations occurred during weeks 27, 47, 79, and 105.

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TABLE 4
Mean Body Weights and Survival of Female Rats in the Lifetime Inhalation Study of Talc

Weeks on Study	0 mg/m ³		6 mg/m ³			18 mg/m ³		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	97	72	101	104	72	98	101	72
2	126	72	127	101	72	125	99	72
3	136	72	139	102	72	138	101	72
4	149	72	144	97	72 ^a	145	97	72
5	153	72	159	104	72	154	100	72
6	160	72	165	103	72	160	101	72
7	165	72	169	102	72	166	101	72
8	168	72	171	102	72	168	100	72
9	174	72	176	101	72	173	100	72
10	178	72	182	102	72	179	101	72
11	181	72	184	102	72	181	100	72
13	186	72	191	103	72	187	101	72
17	194	72	201	104	72	197	101	72
21	206	72	211	103	72	207	101	72
25	213	72	216	101	72	214	100	72
29 ^b	215	68	219	101	69	213	99	69
33	224	68	227	101	69	221	99	69
38	233	68	237	102	69	229	98	69
41	239	68	242	101	69	235	98	69
45	248	68	251	101	69	242	98	69
49 ^b	256	65	259	101	66	252	98	66
53	266	65	270	102	66	260	98	66
57	276	62	277	101	66	269	98	65
61	285	62	288	101	66	276	97	65
65	290	61	288	100	66	277	96	65
69	296	61	292	99	66	281	95	65
73	300	61	295	98	64	284	95	65
77	303	61	297	98	62	284	94	64
81 ^b	300	57	301	100	55	283	94	59
85	306	54	302	99	55	283	93	57
89	307	52	305	99	55	287	94	53
93	307	49	305	99	53	286	93	49
97	303	46	304	100	50	281	93	43
101	291	44	296	102	47	271	93	39
105 ^b	288	37	295	103	43	271	94	33
109	290	32	288	99	28	273	94	26
113	289	24	273	94	24	260	90	23
117	283	18	264	93	18	256	90	21
121	277	13	264	95	14	231	84	13
123	268	13	260	97	13	231	86	10
Terminal sacrifice		12			13			9
Mean for weeks								
1-13	156		159	102		156	100	
14-52	225		229	102		223	99	
53-123	291		288	99		271	93	

^a The number of animals weighed for this week is fewer than the number of animals surviving.

^b Interim evaluations occurred during weeks 27, 47, 79, and 105.

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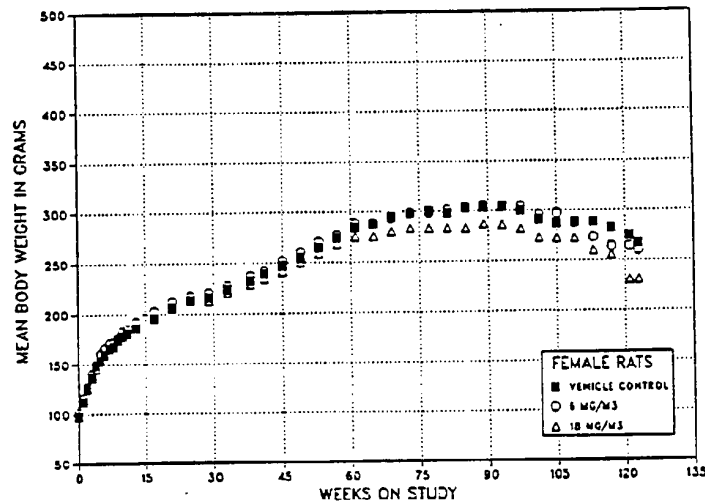
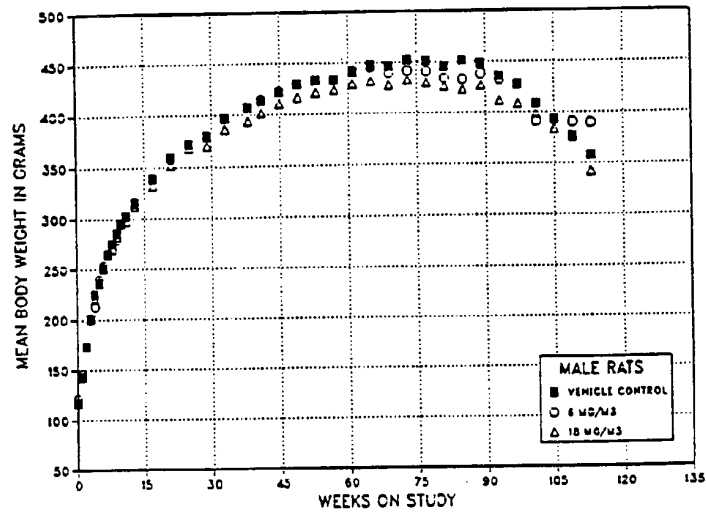


FIGURE 2
Growth Curves for Male and Female Rats Administered Talc by Inhalation for Their Lifetime

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Lung: Absolute and relative lung weights of male rats exposed to 18 mg/m³ were significantly greater than those of controls at the 6-, 11-, and 18-month interim evaluations and at the end of the study, while those of female rats exposed to 18 mg/m³ were significantly greater than those of controls at the 11-, 18-, and 24-month interim evaluations and at the end of the study (Appendix E). Although lung weights of males exposed to 6 mg/m³ were not significantly different from controls at any of the interim evaluations, those of females at the 18-month interim evaluation and at the end of the lifetime study were significantly greater.

Pulmonary lesions in male and female rats occurring in response to the inhalation of talc aerosols were generally similar at the interim evaluations and the end of the study, but varied in incidence, extent, and severity with exposure concentration and duration (Table 5). At necropsy, the lungs of exposed rats had multiple small, round, pale white lesions visible through the visceral pleura. These lesions were generally larger and more extensive in rats exposed to 18 mg/m³ than in those exposed to 6 mg/m³, and at the end of the study than at the earlier interim evaluations.

At the 6-month interim evaluation, the pulmonary lesions consisted of multiple, focal accumulations of alveolar macrophages and infrequent neutrophils within alveolar lumens (inflammation, granulomatous). When viewed under polarized light, the cytoplasm of the alveolar macrophages contained birefringent particles believed to be talc. In two female rats, the alveolar epithelium in some affected areas had increased numbers of low cuboidal type II pneumocytes (alveolar epithelial hyperplasia), but there was no apparent increase in the amount of collagen within the alveolar septa. The peribronchial lymphoid aggregates of several rats also contained focal accumulations of macrophages that varied from a few to approximately 10 cells in the plane of section (peribronchial hyperplasia, histiocytic).

In contrast to the first interim evaluation, hyperplasia of type II pneumocytes was associated with the intra-alveolar accumulations of macrophages in all exposed rats examined at 11 months. Moreover, in the most severely affected foci, the alveolar septa were thickened by the accumulation of reticulin and collagen fibers (interstitial fibrosis). The lesions in rats examined

at 18 and 24 months and in core study rats were similar but generally larger and more extensive (Plates 1 and 2). Although alveolar macrophages predominated in the inflammatory lesions, varying numbers of neutrophils were also present and the interstitium contained infiltrates of mononuclear inflammatory cells (lymphocytes and macrophages). Moreover, epithelioid macrophages and multinucleated giant cells were also seen within foci of inflammation at these later time points. In some rats, there were well-delineated areas of fibrosis that completely obliterated the alveoli (Plates 3 and 4). Hyperplasia of the alveolar epithelium was often prominent at the margins of these lesions. The affected cells were cuboidal or columnar with prominent nucleoli and exhibited some pleomorphism.

In addition to the changes described above, squamous metaplasia of the alveolar epithelium (Plate 5) was observed in two male and eight female rats in the 18 mg/m³ groups of the core study (Table 5). The metaplasia was usually associated with inflammation and was characterized by the replacement of alveolar type I and type II pneumocytes by well-differentiated keratinized squamous cells. Squamous cysts were also seen in three males and seven females in the 18 mg/m³ groups and in one female in the 6 mg/m³ group. The cysts had outer walls of well-differentiated, stratified squamous epithelium without cellular atypia and central lumens often containing sloughed keratin.

Although an alveolar/bronchiolar adenoma was seen in one 6 mg/m³ female at the 18-month interim evaluation, the remainder of the pulmonary neoplasms were seen in rats in the core study (Table 6). The incidences of alveolar/bronchiolar adenoma, carcinoma, and adenoma or carcinoma (combined) in female rats exposed to 18 mg/m³ were significantly greater than those of controls. A squamous cell carcinoma was also observed in an 18 mg/m³ female. Alveolar/bronchiolar neoplasms occurred in two males exposed to talc aerosols, one at each of the exposure concentrations, and none were seen in control males. Because of the low number of affected male rats, these neoplasms could not be attributed to talc exposure.

Because of the moderate to marked hyperplasia of the alveolar epithelium associated with the inflammatory lesions and because of the fibrosis and

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TABLE 5
Incidences of Selected Lung Lesions in Rats in the Lifetime Inhalation Study of Talc

	Male			Female		
	0 mg/m ³	6 mg/m ³	18 mg/m ³	0 mg/m ³	6 mg/m ³	18 mg/m ³
6-Month Interim Evaluation						
Lung ^a	3	3	3	3	3	3
Inflammation, Granulomatous ^b	0	3*(1.3) ^c	3*(2.3)	0	3*(1.3)	3*(3.0)
Peribronchial Hyperplasia, Histiocytic	0	1 (1.0)	2 (2.0)	0	1 (1.0)	2 (1.0)
Hyperplasia, Alveolar Epithelium	0	0	0	0	1 (1.0)	1 (1.0)
11-Month Interim Evaluation						
Lung	2	3	3	3	3	3
Inflammation, Granulomatous	0	3*(1.7)	3*(3.0)	0	3*(1.7)	3*(2.7)
Peribronchial Hyperplasia, Histiocytic	0	0	0	0	1 (1.0)	2 (1.5)
Hyperplasia, Alveolar Epithelium	0	3*(2.0)	3*(1.7)	0	3*(1.0)	3*(2.3)
Interstitial, Fibrosis, Focal	0	2 (1.0)	3*(1.0)	0	2 (1.0)	3*(1.0)
18-Month Interim Evaluation						
Lung	3	3	2	3	3	3
Inflammation, Granulomatous	1 (1.0)	3 (1.3)	2 (2.0)	0	3*(1.7)	3*(2.0)
Peribronchial Hyperplasia, Histiocytic	0	2 (1.0)	2 (1.0)	0	1 (1.0)	2 (1.0)
Hyperplasia, Alveolar Epithelium	1 (1.0)	3 (1.0)	2 (1.0)	1 (1.0)	3 (1.0)	3 (1.3)
Interstitial, Fibrosis, Focal	0	3*(1.0)	2 (1.5)	0	3*(1.3)	3*(1.7)
Alveolar/bronchiolar Adenoma	0	0	0	0	1	0
24-Month Interim Evaluation						
Lung	3	6	2	5	9	3
Inflammation, Granulomatous	0	6*(1.5)	2 (2.0)	1 (1.0)	9**(1.4)	3 (1.7)
Peribronchial Hyperplasia, Histiocytic	0	1 (1.0)	1 (2.0)	0	2 (1.0)	0
Hyperplasia, Alveolar Epithelium	0	6*(1.0)	2 (1.5)	1 (1.0)	9**(1.4)	2 (2.3)
Interstitial, Fibrosis, Focal	0	5*(1.0)	2 (1.5)	0	8**(1.4)	3*(3.0)
Core Study						
Lung	49	50	50	50	48	50
Inflammation, Granulomatous	2 (1.0)	50**(1.6)	49**(2.3)	2 (1.5)	47**(1.5)	50**(2.8)
Peribronchial Hyperplasia, Histiocytic	0	12**(1.3)	8**(1.9)	0	8**(1.3)	9**(1.3)
Alveolar Epithelium, Hyperplasia	5 (2.0)	26**(1.3)	38**(1.7)	2 (1.0)	27**(1.2)	47**(2.1)
Alveolus, Metaplasia, Squamous	0	0	2 (1.0)	0	0	8*(1.1)
Interstitial, Fibrosis, Focal	1 (1.0)	16**(1.2)	33**(1.8)	1 (1.0)	24**(1.5)	44**(2.1)
Cyst (Squamous)	0	0	3	0	1	7**

* Significantly different (P≤0.05) from the control by Fisher's exact test (interim evaluation) or logistic regression (lifetime study)

** P≤0.01

^a Number of animals with lung examined microscopically.

^b Number of animals with lesion.

^c Average severity grades of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

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TABLE 6
Incidences of Lung Neoplasms in Rats in the Lifetime Inhalation Study of Talc

	Male			Female		
	0 mg/m ³	6 mg/m ³	18 mg/m ³	0 mg/m ³	6 mg/m ³	18 mg/m ³
Core Study						
Alveolar/bronchiolar Adenoma						
Overall rates ^a	0/49 (0%)	1/50 (2%)	1/50 (2%)	1/50 (2%)	0/48 (0%)	9/50 (18%)
Terminal rates ^b	0/9 (0%)	0/14 (0%)	1/16 (6%)	0/11 (0%)	0/13 (0%)	1/9 (11%)
First incidence (days)	— ^d	781	799 (T)	805	—	716
Logistic regression ^c	P=0.494	P=0.527	P=0.615	P<0.001	P=0.503N	P=0.010
Alveolar/bronchiolar Carcinoma						
Overall rates	0/49 (0%)	0/50 (0%)	1/50 (2%)	0/50 (0%)	0/48 (0%)	5/50 (10%)
Terminal rates	0/9 (0%)	0/14 (0%)	1/16 (6%)	0/11 (0%)	0/13 (0%)	3/9 (33%)
First incidence (days)	—	—	799 (T)	—	—	828
Logistic regression	P=0.370	— ^e	P=0.615	P=0.003	—	P=0.028
Alveolar/bronchiolar Adenoma or Carcinoma						
Overall rates	0/49 (0%)	1/50 (2%)	1/50 (2%)	1/50 (2%)	0/48 (0%)	13/50 (26%)
Terminal rates	0/9 (0%)	0/14 (0%)	1/16 (6%)	0/11 (0%)	0/13 (0%)	4/9 (44%)
First incidence (days)	—	781	799 (T)	805	—	716
Logistic regression	P=0.494	P=0.527	P=0.615	P<0.001	P=0.503N	P<0.001
Squamous Cell Carcinoma						
Overall rates	0/49 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/48 (0%)	1/50 (2%)

(T) Terminal sacrifice

^a Number of tumor-bearing animals/number of animals examined microscopically.^b Observed incidence at terminal kill^c Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard these lesions as nonfatal. A lower incidence in a dose group is indicated by N.^d Not applicable; no tumors in animal group^e Value of statistic cannot be computed.

inflammation occurring within some of the neoplasms, there was considerable difficulty in determining the biological nature of the proliferative lesions observed and in distinguishing hyperplasia from adenoma and adenoma from carcinoma. The adenomas were irregular, circumscribed masses consisting of cuboidal to columnar epithelium arranged in alveolar, tubular, or papillary formations and separated by varying amounts of collagenous connective tissue. The neoplastic epithelium generally formed a single layer and was relatively uniform with minimal cellular atypia. The carcinomas were distinguished from the adenomas on the basis of having greater cellular pleomorphism and atypia, but they exhibited little evidence of invasion and none metastasized (Plates 6 and 7). In several benign and malignant neoplasms, the central portion of the mass was composed primarily of dense collagen and the epithelial component was

located at the periphery. The extent of fibrosis in these neoplasms is not typical of spontaneous alveolar/bronchiolar neoplasms in control F344/N rats. The fibrous connective tissue was not interpreted as being a primary scirrhous response to the neoplastic epithelium, but rather a component of the prolonged inflammatory reaction to talc.

Lymph node: Histiocytic hyperplasia, consisting of accumulations of macrophages in the subcapsular and medullary sinuses, occurred in the bronchial lymph nodes (male: 0 mg/m³, 0/41; 6 mg/m³, 44/48; 18 mg/m³, 46/49; female: 0/46, 40/47, 45/47) and in the mediastinal lymph nodes (male: 0/48, 40/49, 43/47; female: 0/47, 33/44, 40/47) of rats exposed to talc (Tables A4 and B4). The macrophages had foamy cytoplasm filled with birefringent particles of talc.

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Nose: Hyperplasia of the respiratory epithelium of the nasal mucosa occurred in three male rats exposed to 6 mg/m³ and 14 male rats exposed to 18 mg/m³, but not in the control group (Table A4). The lesion consisted of an increase in the number of goblet cells primarily in the mucosa of the nasal septum. Hyperplasia of the respiratory epithelium also occurred in several female rats, but the incidences in the exposed groups were not significantly increased (Table B4).

During the pathology review process, it was noted that male and female rats in control and exposed groups had large eosinophilic droplets in the cytoplasm of the olfactory and, to a lesser extent, the respiratory epithelium. The lesion (cytoplasmic alteration) was focal or multifocal and usually located near the junction of the two epithelial types. Although present in the controls, the incidences were increased in exposed rats (males: 3/49, 18/48, 40/47; females: 5/48, 23/45, 46/48).

Adrenal medulla: Focal adrenal medulla hyperplasia or pheochromocytoma were observed in rats at the various interim evaluations, but the number of affected rats was too small to draw definitive conclusions. However, in the core study, benign, malignant, or complex (combined) pheochromocytomas occurred with a significant positive trend in male and female rats, and the incidences in the 18 mg/m³ groups were significantly greater than those of controls by pairwise comparisons (Table 7). Moreover, bilateral pheochromocytomas were more frequent in exposed male rats than in controls (Tables A3 and B3). Although adrenal medulla hyperplasia occurred with similar frequency among exposed and control female rats, the incidences of hyperplasia in exposed males were significantly lower than controls. The lower incidences in exposed males are possibly due, in part, to the reduced amount of normal medullary tissue (e.g., medullary tissue without a pheochromocytoma) in which to observe hyperplasia.

Focal hyperplasia and pheochromocytoma constitute a morphological continuum. Focal hyperplasia consisted of irregular, small foci of small to normal sized medullary cells arranged in packets or solid clusters slightly larger than normal; compression of the surrounding tissue was minimal or absent. Pheochromocytomas were generally larger than focal hyperplasia, caused variable compression of the surrounding parenchyma, and many obscured much

or all of any remaining normal medullary tissue. The neoplastic cells were arranged in variably sized aggregates, large solid clusters, and/or trabecular cords several layers thick separated by a delicate fibrovascular stroma. The larger neoplasms usually exhibited greater cellular pleomorphism and atypia than smaller neoplasms. Because the only morphological criteria that unambiguously distinguish malignant from benign pheochromocytomas is frank invasion or metastasis, a diagnosis of malignant pheochromocytoma was made only when there was invasion of the capsule. Complex pheochromocytomas consisted of an admixture of neoplastic pheochromocytes and neuroblasts, ganglion cells, and/or Schwann cells.

Lung Talc Burden

The lung talc burdens of exposed rats, normalized to control lung weight or exposure level, are presented in Tables F2 and F3. The lung talc burden normalized to control lung weight (mg talc/g control lung) adjusts for differences in lung weight between sexes or at different ages. The lung burden normalized to control lung weight and exposure level (mg talc/g control lung/mg/m³) adjusts for exposure level to determine the effect of exposure concentration on talc clearance from the lung.

The data, normalized to control lung weight, show that talc burdens of rats exposed to 6 mg/m³ were similar between males and females and increased progressively from 6 to 24 months (Table F2). Lung talc burdens in females exposed to 18 mg/m³ also increased progressively from 6 to 24 months. In contrast, lung talc burdens of males at the 18 mg/m³ exposure concentration increased from 6 to 18 months, but remained about the same at 18 and 24 months.

The exposure-normalized data show that lung talc burdens were generally proportional to exposure concentration at each interim evaluation. The exposure-normalized lung burdens of rats exposed to 6 or 18 mg/m³ were generally similar at each of the interim evaluations except for slight increases for males at 6 and 11 months and females at 6 months (Table F3). This suggests that either clearance of talc was not substantially impaired by increasing the exposure concentration, or that clearance of talc was impaired similarly at both exposure levels.

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TABLE 7
Incidences of Nonneoplastic Lesions and Neoplasms of the Adrenal Medulla in Rats
in the Lifetime Inhalation Study of Talc

	Male			Female		
	0 mg/m ³	6 mg/m ³	18 mg/m ³	0 mg/m ³	6 mg/m ³	18 mg/m ³
11-Month Interim Evaluation						
Adrenal Medulla ^a	2	3	3	3	3	3
Hyperplasia ^b	0	0	0	0	0	0
Pheochromocytoma, Benign	1	0	0	0	0	0
18-Month Interim Evaluation						
Adrenal Medulla	3	3	2	2	3	3
Hyperplasia	0	1 (1.0) ^c	0	0	1 (2.0)	1 (2.0)
Pheochromocytoma, Benign	0	0	1	0	0	0
24-Month Interim Evaluation						
Adrenal Medulla	3	6	2	5	9	3
Hyperplasia	2 (1.5)	2 (2.0)	0	3 (2.0)	0	0
Pheochromocytoma, Benign	0	2	0	0	4	0
Pheochromocytoma, Benign, Bilateral	1	1	2	0	1	3
Core Study						
Adrenal Medulla	49	48	47	48	47	49
Hyperplasia	20 (2.7)	8** (2.3)	9* (3.2)	22 (2.5)	20 (2.2)	16 (2.6)
Pheochromocytoma, Benign						
Overall rates ^d	25/49 (51%)	30/48 (63%)	36/47 (77%)	13/48 (27%)	14/47 (30%)	18/49 (37%)
Terminal rates ^e	6/9 (67%)	11/14 (79%)	16/16 (100%)	5/11 (45%)	5/13 (38%)	6/9 (67%)
First incidence (days)	429	558	614	678	705	697
Logistic regression test ^f	P=0.007	P=0.213	P=0.009	P=0.185	P=0.541	P=0.225
Pheochromocytoma, Malignant						
Overall rates	3/49 (6%)	3/48 (6%)	7/47 (15%)	0/48 (0%)	1/47 (2%)	10/49 (20%)
Terminal rates	1/9 (11%)	1/14 (7%)	3/16 (19%)	0/11 (0%)	0/13 (0%)	3/9 (33%)
First incidence (days)	670	544	645	— ^g	849	784
Logistic regression test	P=0.096	P=0.662	P=0.178	P<0.001	P=0.509	P=0.001
Pheochromocytoma, Complex						
Overall rates	0/49 (0%)	2/48 (4%)	1/47 (2%)	0/48 (0%)	0/47 (0%)	0/49 (0%)
Terminal rates	0/9 (0%)	1/14 (7%)	0/16 (0%)	0/11 (0%)	0/13 (0%)	0/9 (0%)
First incidence (days)	—	558	743	— ^h	—	—
Logistic regression test	P=0.486	P=0.230	P=0.503	— ^h	—	—
Pheochromocytoma, Benign, Malignant, or Complex						
Overall rates	26/49 (53%)	32/48 (67%)	37/47 (79%)	13/48 (27%)	14/47 (30%)	23/49 (47%)
Terminal rates	7/9 (78%)	12/14 (86%)	16/16 (100%)	5/11 (45%)	5/13 (38%)	8/9 (89%)
First incidence (days)	429	544	614	678	705	697
Logistic regression test	P=0.007	P=0.147	P=0.006	P=0.014	P=0.541	P=0.024

* Significantly different (P≤0.05) from the control by logistic regression

** P≤0.01

^a Number of animals with adrenal medulla examined microscopically.

^b Number of animals with lesion.

^c Average severity grades of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

^d Number of animals with neoplasm per number of rats with adrenal medulla examined microscopically.

^e Observed incidence at terminal kill

^f Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard these lesions as nonfatal.

^g Not applicable; no tumors in animal group

^h Value of statistic cannot be computed.

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Pulmonary Function

Results of the respiratory function measurements are presented in Tables F9 through F41. A progressive dose and time-related impairment of respiratory function was observed in both male and female rats exposed to talc. The impairment was restrictive in nature, consisting of reduced lung volume, increased lung stiffness, reduced gas exchange efficiency, and nonuniform intrapulmonary gas distribution.

6-Month Interim Evaluation: At 6 months there were few significant differences between values for rats exposed to 18 mg/m³ and controls, and no significant differences between values for rats exposed to 6 mg/m³ and controls. There were, however, slight trends among both males and females toward smaller lung volumes and reduced forced expiratory flow. Total lung capacity, vital capacity, and forced vital capacity were all slightly smaller in the 18 mg/m³ groups, but only the forced vital capacity of females differed significantly from controls. All forced expiratory flow rates were lower in the 18 mg/m³ groups, but only those of males were significantly lower than those of the controls. The reduced flow rates were partly related to the smaller lungs, but even volume-normalized flow tended to be reduced in the exposed rats. The reduced flow rates most likely reflected changes in small airways. Total pulmonary resistance, which primarily reflects flow resistance in large airways, was unaffected.

11-Month Interim Evaluation: Functional alterations were clearly apparent in exposed males and females after 11 months. Total lung capacity, vital capacity, and forced vital capacity were significantly lower in males and females exposed to 18 mg/m³ and males exposed to 6 mg/m³. The reduced volume was accompanied by significant reductions in quasistatic lung compliance in males, and both dynamic and quasistatic lung compliance in females. The volume and compliance changes indicate a stiffening of the lung (or increase in elastic recoil). Forced expiratory flows during mid to late expiration were slightly lower in exposed males than in controls, but the differences were not significant.

A reduction of alveolar-capillary gas exchange efficiency was reflected by a significant reduction of carbon monoxide diffusing capacity in the 18 mg/m³ male and female rat groups. Although diffusing capacity is somewhat volume dependent, the reduced lung volume did not completely account for the

change. Volume-normalized diffusing capacity was also significantly reduced in male and female rats exposed to 18 mg/m³.

18-Month Interim Evaluation: Total lung capacity, vital capacity, and forced vital capacity of all exposed groups of male and female rats were significantly lower than those of controls at 18 months, except for vital capacity of males exposed to 6 mg/m³. In females exposed to 18 mg/m³, these decreases were accompanied by significant increases in resting (functional residual capacity) and minimum (residual) volumes. The decrease in volume at maximum inflations (total capacity, vital capacity, and forced vital capacity) reflected the inability of the stiffened lungs to stretch normally. Volume-normalized forced expiratory flows of exposed male and female rats were generally greater than those of controls, due to the reduced lung volume and little or no reduction in flow.

All parameters of lung compliance in male and female rats exposed to 18 mg/m³ were also significantly lower than controls at 18 months, while two of the three compliance parameters were significantly lower at the 6 mg/m³ exposure level. The carbon monoxide diffusing capacities in males and females exposed to 18 mg/m³ were significantly lower than controls at 18 months, which is consistent with the findings at 11 months.

The slope of phase III of the single-breath N₂ washout of male and female rats exposed to 18 mg/m³ was significantly greater than controls, apparently due to uneven mixing of oxygen with residual nitrogen in the lung during maximal inflation. This finding reflects a nonuniform distribution of inhaled air.

24-Month Interim Evaluation: Because of reduced survival in all groups of male and female rats, fewer animals remained alive at 24 months for evaluation of pulmonary function. Because of the smaller group sizes (3 rats each from the control and 18 mg/m³ groups were evaluated), few of the differences were statistically significant. Nevertheless, there were reductions in lung volume parameters (total lung capacity, vital capacity, and forced vital capacity), lung compliance, and carbon monoxide diffusing capacity in exposed male and female rats consistent with the findings at the earlier time periods.

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The progression of the functional impairments over the course of the study are illustrated in Figure 3, which plots the data for three functional parameters obtained from the 3 male and 3 female rats in the 18 mg/m³ exposure groups surviving until 24 months.

Bronchoalveolar Lavage and Lung Biochemistry

Following the completion of the pulmonary function tests at the 24-month interim evaluation, bronchoalveolar lavage was performed on the remaining rats in these groups and the lavage fluid was evaluated for enzymes, protein, and cell content as shown in Tables F4 and F5. Values for glucose-6-phosphate dehydrogenase and glutathione peroxidase are not reported because they were below the limits of detection.

The values for β -glucuronidase, alkaline phosphatase, lactate dehydrogenase, and total protein in both male and female rats exposed to 18 mg/m³ talc were significantly greater than those of controls. In addition, females in this group had a significantly higher value for glutathione reductase. Both male and female rats exposed to 6 mg/m³ talc had significantly greater β -glucuronidase values, but only female rats exposed to 6 mg/m³ had higher values of alkaline phosphatase, lactate dehydrogenase, and protein. The percentages of polymorphonuclear leukocytes in the lavage fluid were also significantly greater in male and female rats exposed to talc at both concentration levels. The increase in enzymes, total protein, and leukocytes are consistent with the morphological findings of a chronic active

inflammatory process and cellular degenerative changes.

The viability and phagocytic activity of alveolar macrophages recovered from the lungs of rats exposed to 6 or 18 mg/m³ talc or from the chamber controls ranged from approximately 60% to 80%. Neither the viability or phagocytic activity were significantly affected by exposure to talc (Table F6).

Table F7 summarizes the effects of talc exposure on collagen metabolism and protein synthesis. Collagenous peptides in lavage fluid and collagen production (% newly synthesized protein) from female rats, but not males, exposed to 6 or 18 mg/m³ were significantly greater than controls. Total lung collagen from males and females at both exposure levels were also significantly greater. Values for non-collagenous protein synthesis were significantly greater in males exposed to 6 or 18 mg/m³ and in females exposed to 18 mg/m³ than in controls.

Lung proteinase activity, as determined from lavage fluid and homogenate supernatant fluid, is shown in Table F8. Acid proteinase activity, primarily cathepsin D, was significantly greater in both males and females exposed to 6 or 18 mg/m³ than in controls. Neutral proteinase activity in homogenate supernatant fluid was also greater in rats exposed to talc. The activity was mostly serine proteinase, like that of polymorphonuclear leukocyte elastase and cathepsin G.

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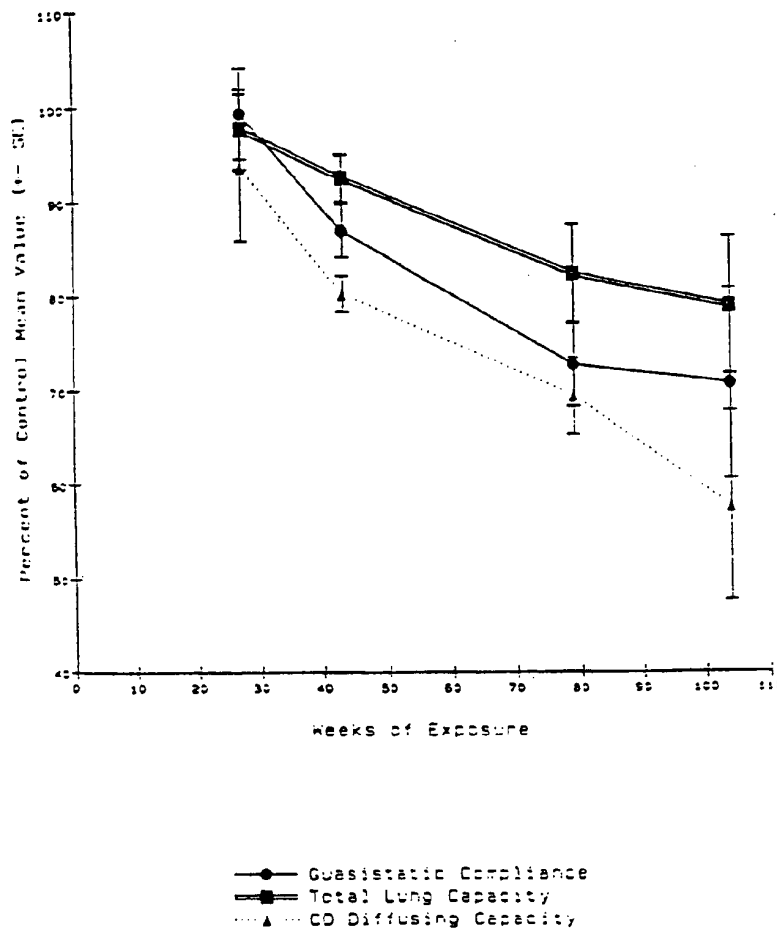


FIGURE 3
Effect of 18 mg/m³ Talc Exposure on Respiratory Function of Male and Female Rats
Surviving to 104 Weeks

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MICE

4-WEEK STUDY DOSE SELECTION

Selection of 6 or 18 mg talc/m³ as the exposure concentrations was based on the results of a 4-week inhalation study in B6C3F₁ mice to determine lung talc burden and histopathologic changes associated with talc exposure. These studies indicated that the amount of talc retained in the lung was similar between sexes and proportional to exposure concentration (Appendix K). Microscopic examination of the lungs revealed an accumulation of alveolar macrophages in the lungs only at 18 mg/m³. Based on these findings it was predicted that aerosol concentrations greater than 18 mg/m³ would overwhelm lung clearance mechanisms, impair lung function, and possibly shorten survival.

2-YEAR STUDY

Survival

Estimates of survival probabilities for male and female mice are shown in Table 8 and in the Kaplan-Meier curves in Figure 4. Survival of male and female mice exposed to talc was similar to that of the controls throughout most of the study. One female mouse exposed to 18 mg/m³ died on day 20 and six others died on day 28 of the study of undetermined cause.

Body Weights and Clinical Findings

Mean body weights of male and female mice exposed to talc were similar to controls throughout the study (Tables 9 and 10, and Figure 5). There were no clinical findings in exposed mice that could be attributed to exposure to talc.

Prior to the start of the study and after 6 months of exposure, serological tests were negative for all viruses tested and *Mycoplasma spp.* At 12 months, 8/24 mice were positive for mouse hepatitis virus (MHV), but retesting of the serum by the enzyme linked immunosorbent assay (ELISA) showed all to be negative. At the end of the study, 7/30 were positive for *Mycoplasma arthritidis* and 21/30 were positive for epizootic diarrhea of infant mice (EDIM). No clinical signs or gross or microscopic evidence of disease associated with *M. arthritidis* was observed. EDIM does not cause clinical disease or pathology in adult mice.

Pathology and Statistical Analyses of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplastic or nonneoplastic lesions of the lung, lymph node, and nose. Summaries of the incidences of nonneoplastic lesions and neoplasms, the individual animal neoplasm diagnoses, and the statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one group are presented in Appendix C for male mice and Appendix D for female mice.

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TABLE 8
Survival of Mice in the 2-Year Inhalation Study of Talc

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Core Study Groups			
Animals initially in study	50	50	50
Natural deaths	16	18	14
Moribund kills	1	2	3
Missing ^a	2	1	1
Missexed ^a	1	1	0
Animals surviving to study termination	30	28	32
Percent survival at end of study ^b	65	58	66
Mean survival (days) ^c	648	648	645
Survival analysis ^d	P=0.886N	P=0.771	P=1.000N
Special Study Groups^a			
Animals initially in study	39	40	40
Natural deaths	4	5	7
Moribund kills	0	1	1
Missing ^a	0	1	1
Scheduled sacrifice	35	33	31
Females			
Core Study Groups			
Animals initially in study	50	50	50
Natural deaths	17	21	21
Moribund kills	2	4	4
Missing ^a	1	1	0
Culled ^a	0	1	0
Animals surviving to study termination	30	23	25
Percent survival at end of study ^b	62	48	50
Mean survival (days) ^c	663	648	590
Survival analysis ^d	P=0.321	P=0.322	P=0.286
Special Study Groups^a			
Animals initially in study	39	40	40
Natural deaths	7	5	10
Moribund kills	2	5	1
Scheduled sacrifice	30	30	29

^a Censored from survival analyses^b Kaplan-Meier determinations^c Mean of all deaths (uncensored, censored, and terminal sacrifice).^d The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns. A negative trend or lower mortality in a dose group is indicated by N.

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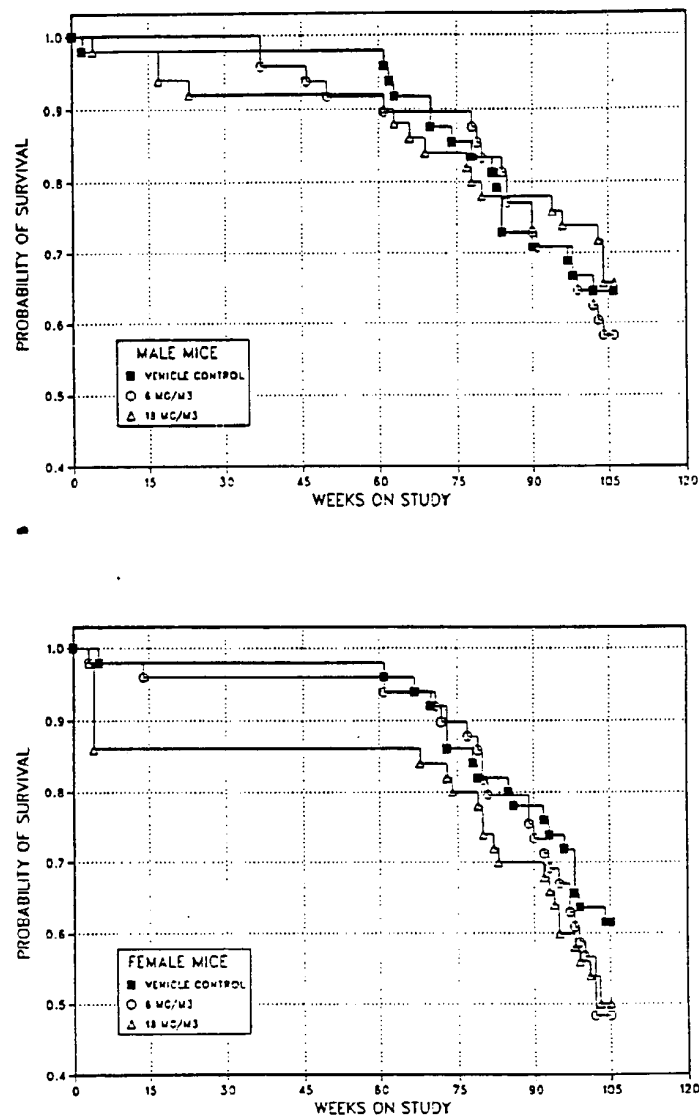


FIGURE 4
Kaplan-Meier Survival Curves for Male and Female Mice Administered Talc by Inhalation for 2 Years

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TABLE 9
Mean Body Weights and Survival of Male Mice in the 2-Year Inhalation Study of Talc

Week on Study	0 mg/m ³		6 mg/m ³		Number of Survivors	18 mg/m ³		Number of Survivors
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)		Av. Wt. (g)	Wt. (% of controls)	
1	23.3	50	23.8	102	50	23.7	102	50
2	24.0	48	23.9	100	49	24.3	101	50
3	25.0	47	25.4	102	49	24.8	99	50
4	25.4	47	26.4	104	49	25.0	98	50
5	26.1	47	26.2	100	49	26.6	102	49
6	27.3	47	27.4	100	49	26.9	99	49
7	27.8	47	27.4	99	49	27.5	99	49
8	25.8	47	27.9	108	49	29.7	115	49
9	28.1	47	28.3	101	48	28.5	101	49
10	28.8	47	28.5	99	48	28.7	100	49
11	29.1	47	29.5	101	48	28.3	97	49
12	29.0	47	29.2	101	48	28.7	99	49
13	30.1	47	30.5	101	48	29.8	99	49
17	31.5	47	30.8	98	48	31.0	98	47
21	32.2	47	30.9	96	48	31.4	98	47
25	33.4	47	31.8	95	48	32.5	97	46
29	33.0	47	32.3	98	48	32.7	99	46
33	33.9	47	33.3	98	48	33.2	98	46
37	34.7	47	34.2	99	46	33.8	97	46
42	35.7	47	35.4	99	46	34.7	97	46
45	36.9	47	36.0	98	46	35.7	97	46
49	36.4	47	35.5	98	45	35.5	98	46
53	36.4	47	36.6	101	44	36.3	100	46
57	36.9	47	35.8	97	44	35.7	97	46
61	36.8	46	37.6	102	43	36.6	100	45
65	37.2	44	37.1	100	43	36.4	98	44
69	36.5	44	37.1	102	43	36.0	99	42
73	37.2	42	36.5	98	43	35.1	94	42
77	36.9	41	35.1	95	43	35.0	95	42
81	37.6	40	36.8	98	40	35.2	94	39
85	37.0	35	37.1	100	37	35.2	95	39
89	36.7	35	35.9	98	37	34.8	95	38
93	34.9	34	36.3	104	34	33.4	96	38
97	34.2	33	35.2	103	34	33.3	97	36
101	33.9	31	34.1	101	31	33.3	98	36
Terminal sacrifice		30			28			32
Mean for weeks								
1-13	26.9		27.3	101		27.1	101	
14-52	34.2		33.4	98		33.4	98	
53-101	36.3		36.2	100		35.1	97	

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TABLE 10
Mean Body Weights and Survival of Female Mice in the 2-Year Inhalation Study of Talc

Week on Study	0 mg/m ³		6 mg/m ³			18 mg/m ³		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	19.3	50	19.3	100	50	19.6	102	50
2	19.9	50	20.1	101	50	20.5	103	50
3	21.0	50	21.3	101	50	21.1	101	50
4	22.4	50	22.5	100	49	21.5	96	49
5	22.5	49	22.7	101	49	23.2	103	43
6	24.4	49	23.7	97	49	23.8	98	43
7	24.6	49	24.5	100	49	24.3	99	43
8	22.1	49	24.2	110	49	26.8	121	43
9	24.6	49	24.9	101	49	25.2	102	43
10	25.2	49	25.4	101	49	25.3	100	43
11	25.6	49	26.2	102	49	25.0	98	43
12	25.5	49	25.1	98	49	25.2	99	43
13	26.3	49	26.4	100	49	25.9	99	43
17	27.5	49	26.7	97	47	27.3	99	43
21	28.4	49	27.2	96	47	27.7	98	43
25	29.5	49	28.1	95	47	28.9	98	43
29	29.8	49	28.6	96	47	28.9	97	43
33	30.1	49	29.7	99	47	29.5	98	43
37	30.7	49	29.9	97	47	29.9	97	43
42	31.7	49	30.8	97	47	30.3	96	43
45	32.4	49	31.7	98	47	31.1	96	43
49	32.2	49	31.2	97	47	31.0	96	43
53	32.7	49	31.4	96	47	31.9	98	43
57	32.7	49	31.0	95	47	31.2	95	43
61	33.1	49	32.9	99	46	32.3	98	43
65	33.0	48	32.4	98	46	32.7	99	43
69	32.7	47	32.4	99	46	32.1	98	42
73	32.8	43	32.1	98	44	31.0	95	41
77	32.6	43	31.3	96	43	31.3	96	40
81	33.5	41	32.7	98	39	32.1	96	37
85	32.5	40	33.0	102	39	32.7	101	35
89	32.7	39	32.1	98	36	32.1	98	35
93	31.7	37	31.7	100	33	31.2	98	33
97	31.5	35	31.7	101	30	30.6	97	30
101	31.8	31	31.4	99	27	31.0	98	27
Terminal sacrifice		30			23			25
Mean for weeks								
1-13	23.3		23.6	101		23.6	101	
14-52	30.3		29.3	97		29.4	97	
53-101	32.6		32.0	98		31.7	97	

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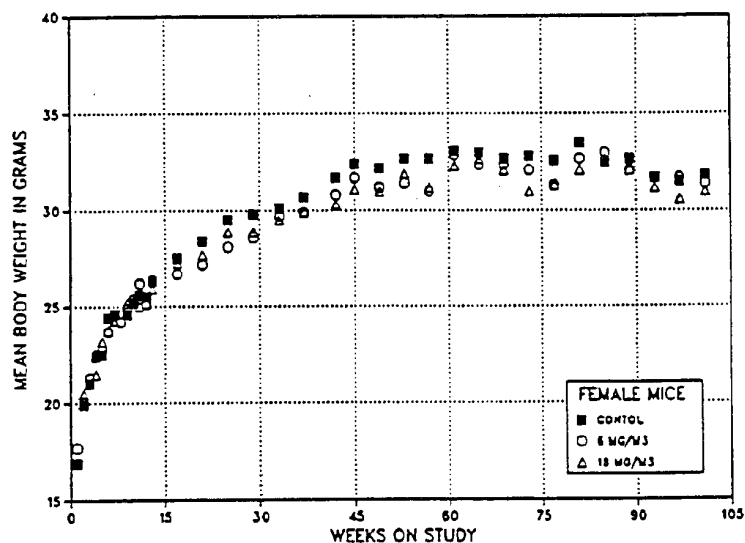
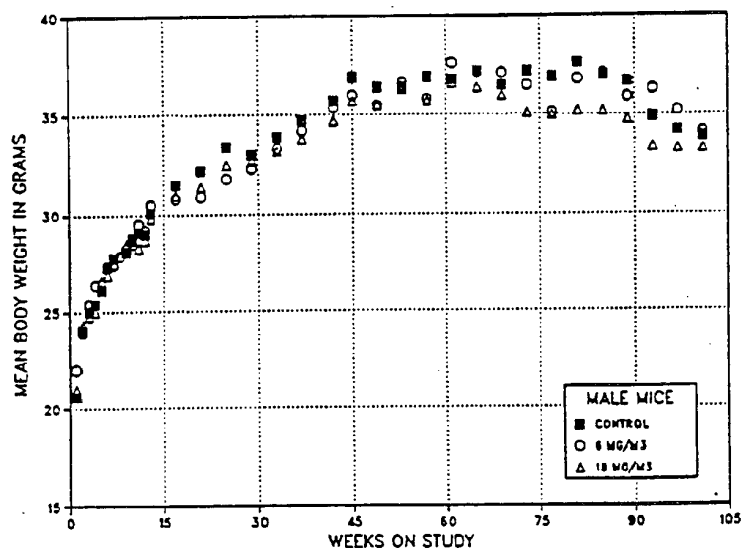


FIGURE 5
Growth Curves for Male and Female Mice Administered Talc by Inhalation For 2 Years

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Lung: Absolute and relative lung weights of male and female mice exposed to 18 mg/m³ talc were significantly greater at the 12- and 18-month interim evaluations and at the end of the study. Lung weights of mice exposed to 6 mg/m³ were similar to controls at each of the interim evaluations.

The pulmonary lesions in mice exposed to talc were similar at the interim evaluations and at the end of the study, but the lesions varied in extent and severity with exposure concentration and duration (Table 11). The principal lung lesion occurring in exposed mice was an accumulation of alveolar macrophages in the alveoli surrounding terminal bronchioles (hyperplasia, macrophage) (Plate 8). The macrophages had abundant, slightly foamy to granular, eosinophilic cytoplasm containing birefringent talc particles. Small numbers of neutrophils were sometimes observed in the affected areas, and the interstitium contained infiltrates of mononuclear inflammatory cells (inflammation, chronic active) (Plates 9 and 10). In contrast to the pulmonary lesions in rats, hyperplasia of type II pneumocytes or fibrosis were not prominent components of the lesions in mice. The incidences of pulmonary neoplasms were similar among exposed groups and controls.

Lymph node: The bronchial lymph nodes of mice exposed to talc contained accumulations of macrophages in the medullary sinuses (hyperplasia, histocyte - male: 0 mg/m³, 1/32; 6 mg/m³, 32/39; 18 mg/m³, 42/44; female: 0/38, 25/37, 39/43; Tables C4 and D4). The macrophages had abundant, slightly foamy to granular, eosinophilic cytoplasm filled with birefringent particles of talc.

Nose: The incidences of focal cytoplasmic alteration were increased in groups of mice exposed to talc (male: 5/45, 23/46, 40/47; female: 29/46, 37/46, 40/50; Tables C4 and D4). Focal cytoplasmic alteration was characterized by the formation of large eosinophilic droplets in the cytoplasm of olfactory and respiratory epithelial cells and was similar to that observed in rats.

Lung Talc Burden

The lung talc burdens, normalized to control lung weight or exposure level, are presented in Tables G2 and G3. Lung talc burden normalized to control lung weights (mg talc/g control lung) adjusts for differences in lung weight between sexes or at different ages. The lung burden normalized to

control lung weight and exposure level (mg talc/g control lung/mg/m³) adjusts for exposure level to determine the effect of exposure concentration on talc clearance from the lung.

The data, normalized to control lung weight, show that talc burdens of mice exposed to 6 mg/m³ were similar between males and females and increased progressively from 6 to 24 months, except for males at 18 months (Table G2). However, because of the small sample size of males at 18 months (two animals), the lung talc burden of this sample may not be representative of the group as a whole. The lung talc burdens of mice exposed to 18 mg/m³ were also similar between sexes at each interim evaluation. Although the talc burdens of males and females increased substantially from 6 to 24 months, the values at 12 and 18 months were similar.

The exposure-normalized data show that lung talc burdens of mice exposed to 18 mg/m³ were disproportionately greater than those of mice exposed to 6 mg/m³ (Table G2). The slight increases in exposure-normalized lung talc burden were statistically significant in males and females at 12 and 24 months, but not at 6 or 18 months. The lack of statistical significance at 18 months might be explained, in part, by the small sample size. These data suggest that clearance of talc from the lung was impaired, or impaired to a greater extent, in mice exposed to 18 mg/m³ than in mice exposed to 6 mg/m³.

Bronchoalveolar Lavage and Lung Biochemistry

Bronchoalveolar lavage was performed and lung homogenate supernatants collected for analyses at 6, 12, 18, and 24 months. A summary of the changes occurring in bronchoalveolar fluid enzymes, protein and cells are shown in Tables G4 through G22. Values for glucose-6-phosphate dehydrogenase, glutathione peroxidase, and alkaline phosphatase were not reported because they were below the limit of detection.

β -Glucuronidase activity of lavage fluid from male and female mice exposed to 18 mg/m³ was greater than that of controls at 12, 18, and 24 months, but not at 6 months. In mice exposed to 6 mg/m³, β -glucuronidase activity was greater than that of controls only at the 24-month interim evaluation. Lactate dehydrogenase and glutathione reductase activities in male and female mice exposed to

TABLE 11
Incidences of Nonneoplastic Lesions and Neoplasms in the Lung of Mice
in the 2-Year Inhalation Study of Talc

	Male			Female		
	0 mg/m ³	6 mg/m ³	18 mg/m ³	0 mg/m ³	6 mg/m ³	18 mg/m ³
6-Month Interim Evaluation						
Lung ^a	4	4	4	4	4	4
Hyperplasia, Macrophage ^b	0	3 (1.0) ^c	4*(1.0)	0	0	4*(1.0)
Inflammation, Chronic Active	0	0	1 (1.0)	0	0	0
12-Month Interim Evaluation						
Lung	4	4	4	3	4	4
Hyperplasia, Macrophage	0	4*(1.0)	4*(1.8)	0	4*(1.0)	4*(2.0)
Inflammation, Chronic Active	0	0	2 (2.0)	0	0	1 (3.0)
18-Month Interim Evaluation						
Lung	4	4	4	4	4	4
Hyperplasia, Macrophage	0	4*(1.3)	4*(2.5)	0	4*(1.3)	4*(2.5)
Inflammation, Chronic Active	0	0	2 (1.5)	0	0	0
Alveolar/bronchiolar Adenoma	0	1	0	1	0	0
Alveolar/bronchiolar Carcinoma	1	0	0	0	0	0
2-Year Study						
Lung	45	47	48	46	48	50
Hyperplasia, Macrophage	3 (2.3)	46**(1.4)	48**(2.8)	2 (2.5)	45**(1.6)	43**(2.8)
Inflammation, Chronic Active	0	16**(1.1)	40**(2.2)	0	25**(1.4)	38**(2.3)
Alveolar Epithelium, Hyperplasia	1 (1.6)	0	0	0	0	1 (1.0)
Alveolar/bronchiolar Adenoma						
Overall rate ^d	6/45 (13%)	4/47 (9%)	9/48 (19%)	3/46 (7%)	2/49 (4%)	2/50 (4%)
Logistic regression ^e	P=0.251	P=0.411N	P=0.371	P=0.467N	P=0.499N	P=0.515N
Alveolar/bronchiolar Carcinoma						
Overall rate	7/45 (16%)	2/47 (4%)	2/48 (4%)	2/46 (4%)	4/49 (8%)	1/50 (2%)
Logistic regression	P=0.069N	P=0.073N	P=0.070N	P=0.325N	P=0.356	P=0.500N
Alveolar/bronchiolar Adenoma or Carcinoma						
Overall rate	12/45 (27%)	5/47 (11%)	11/48 (23%)	5/46 (11%)	6/49 (12%)	3/50 (6%)
Logistic regression	P=0.522N	P=0.043N	P=0.423N	P=0.269N	P=0.519	P=0.367N

* Significantly different (P≤0.05) from the control by Fisher's exact test (interim evaluation) or logistic regression (2-year study)

** P≤0.01

^a Number of animals with lung examined microscopically.

^b Number of animals with lesion.

^c Average severity grades of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

^d Number of animals with neoplasm per number of mice examined microscopically.

^e Beneath the controls incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the control and that dosed group. The logistic regression tests regard these lesions as nonfatal. A negative trend or a lower incidence in a dosed group is indicated by N.

Results

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18 mg/m³ were significantly greater than those of controls at 18 and 24 months. Glutathione activity of males exposed to 18 mg/m³ was also greater than controls at 12 months. Values for total protein in lavage fluid from males and females in the 18 mg/m³ groups were significantly greater than controls at 18 months; at 24 months only that of males was significantly greater.

Significant differences in total and differential cell counts between exposed and control mice were observed only at 18 and 24 months at the high concentration level (Tables G8 to G11). The numbers of total nucleated cells, polymorphonuclear leukocytes, and macrophages were significantly greater in males and females exposed to 18 mg/m³ than in controls. Exposure of mice to 6 or 18 mg/m³ talc produced a concentration-related decrease in phagocytic activity of macrophages derived from lavage fluid (Tables G12 to G14). The number of macrophages containing phagocytized sheep erythrocytes from male and female mice exposed to 18 mg/m³ was significantly lower than that from control mice at 12, 18, and 24 months. Although phagocytic activity of macrophages from mice exposed to 6 mg/m³ was intermediate between controls and the high concentration groups, only the difference between the exposed and control males at 12 months was statistically significant.

The effects of talc exposure on lavage fluid collagenous peptides and total lung collagen are shown in Tables G15 through G18. The amount of collagenous peptides in lavage fluid from male mice exposed to 18 mg/m³ was significantly greater than that of controls at 12, 18, and 24 months, while collagenous peptides of females exposed to 18 mg/m³ were significantly increased only at 24 months. Consistent with these findings, total lung collagen was significantly greater in male mice at the high exposure concentration at 18 and 24 months and in females at 24 months. Collagenous peptides and total lung collagen from mice exposed to 6 mg/m³ were similar to controls at each of the interim evaluations.

The acid and neutral proteinase activity of lung homogenate supernatant fluid and the acid proteinase activity of lavage fluid are shown in Tables G19 through G22. Although there were no consistent exposure-related changes in lavage fluid acid proteinase activity at any of the interim evaluations, acid proteinase activity in supernatant fluid from male and female mice exposed to 18 mg/m³ was significantly greater than controls at 12, 18, and 24 months. The increase in acid proteinase activity was primarily due to cathepsin D-like activity. There were no consistent exposure-related changes in neutral proteinase activity at any of the interim evaluations.

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Talc, NTP TR 421

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DISCUSSION AND CONCLUSIONS

Talc ore may contain several other minerals including calcite, dolomite, magnesite, tremolite, anthophyllite, antigorite, quartz, pyrophyllite, micas, or chlorites. Since talc products are sold in a multitude of grades which have physical or functional characteristics especially suited for particular applications, occupational and consumer exposures to talc are complex. Exposure to industrial grade talc is known to cause pulmonary fibrosis, but the limited data on exposure to cosmetic grade talc are conflicting. Recently, epidemiology studies have revealed an association between nonfibrous talc and lung cancer risk (Thomas and Stewart, 1987). Talc was nominated by NIOSH for study by the NTP because of widespread human exposure and because of the lack of adequate information on its chronic toxicity and potential carcinogenicity.

The NTP toxicology and carcinogenicity studies of non-asbestiform, cosmetic grade talc, a finely powdered hydrous magnesium silicate, were conducted by exposing groups of male and female F344/N rats and B6C3F₁ mice to target aerosol concentrations of 0, 6 or 18 mg/m³ talc for 6 hours daily, 5 days per week. Rats were exposed to talc until mortality in any group reached 80% (113 weeks for males and 122 weeks for females). Mice were exposed for 103 or 104 weeks. Exposure concentrations for the long-term studies were based on talc deposition and clearance patterns obtained from 4-week inhalation studies (Hanson *et al.*, 1985). In these studies, the amount of talc retained per unit of lung tissue was 79, 190, or 840 µg/g for male rats and 76, 185, or 770 µg/g for female rats exposed to 2, 6, or 18 mg/m³. The amount of talc retained per unit of lung tissue in mice exposed at the same concentration levels were 130, 330, or 1,140 µg/g for males and 110, 330, or 1,160 µg/g for females. Only rats and mice at the highest exposure level had talc-containing macrophages within the alveolar spaces. Because there was a direct relationship between chamber concentration and lung talc burden and because of histologic evidence of a talc accumulation in alveolar macrophages at the 18 mg/m³ concentration, it was predicted that higher levels would overwhelm lung clearance mechanisms in both species and cause deterioration of lung functions.

Thus, 18 mg/m³ was chosen as the top exposure concentration for the long-term studies.

The overall mean chamber concentrations achieved in the NTP long-term studies were 6.1 and 18.6 mg/m³ for the rat study and 5.9 and 16.7 mg/m³ for the mouse study. The average mass mean aerodynamic diameter of the talc particles was calculated to be 2.7 µm and 3.2 µm for the 6 and 18 mg/m³ rat chambers and 3.3 µm and 3.6 µm for the 6 and 18 mg/m³ mouse chambers, respectively. Seventy-five percent of the talc particles counted in four samples were in the 1 to 3 µm range. It has been shown, using aerosols of monodisperse aluminosilicate particles, in rats that particles larger than 10 µm are nearly all removed by inertial impaction in the nasal chamber or at bifurcation of the airways, while the percentage of particles deposited in the alveolar ducts and alveoli rises from almost zero at 10 µm to about 10% at about 1 µm (Raabe *et al.*, 1977). Thus, the large proportion of talc particles in these NTP studies were in the respirable range.

Because of difficulties with the aerosol concentration monitoring system for the 18 mg/m³ rat chamber, there was a 7-week period beginning at study week 11 during which the chamber concentration for the high-dose rats varied from approximately 30 to 40 mg/m³. Further, there was a 12-week period beginning at approximately week 70 during which there were difficulties in generating the talc aerosol and the chamber concentrations for rats and mice were substantially lower than the target concentrations (Figures H5 to H8). Although the exposure concentrations varied substantially from target concentrations during these periods, this does not preclude drawing conclusions regarding the chronic toxicity and carcinogenicity of talc. Since talc is a relatively inert particle, the amount of talc deposited and retained at the target site (lung talc burden) is a more relevant measure of talc exposure than chamber concentration. The problems with maintaining the target concentrations in the NTP studies did not have any apparent substantive effect on lung talc burdens.

The lung talc burden represents the difference between the amount of talc deposited in the lung and the amount removed by the clearance mechanisms. Inhaled particles deposited on the mucosal surface of the trachea, bronchi, or bronchioles are transported up the airways and from the lung through the ciliary activity of the respiratory epithelium, while particles reaching the alveolar region are phagocytized by alveolar macrophages and, to a lesser extent, other phagocytic inflammatory cells. Some of the alveolar macrophages migrate to the ciliated epithelium of the airways while others cross the alveolar lining to enter the interstitium and finally the lymphatics. Phagocytic cells reaching the lymphatics are transported in the lymph to the bronchial and mediastinal lymph nodes. Depending on the physiochemical properties of the inhaled particles, they may be partially or completely broken down within phagolysosomes of the macrophages and soluble components released from the cell. Talc is insoluble in water, cold acids, and alkalis and is likely to be insoluble in biological fluids. Talc particles were observed within macrophages in the lung and bronchial and mediastinal lymph nodes of rats and mice in these inhalation studies.

The lung talc burden of rats was greater than that of mice at each of the exposure concentrations and interim evaluations. The difference in lung talc burden is most likely related to anatomical and physiological differences known to influence particle deposition and retention including air flow pattern and velocity, respiratory rate, tidal volume, and clearance rate (McMahon *et al.*, 1977; Raabe *et al.*, 1977). The lung talc burdens of exposed rats and mice were generally similar between males and females at each exposure concentration and increased progressively with exposure duration. This indicated that the amount of talc deposited in the lung exceeded the clearance from the lung. The lung talc burden of rats was also generally proportional to exposure concentration at each interim evaluation, indicating that clearance of talc was not substantially impaired by increasing the exposure concentration, or that clearance of talc was impaired similarly at both exposure levels. In contrast, the lung talc burden of mice exposed to 18 mg/m³ was disproportionately greater than that of mice exposed to 6 mg/m³, indicating that clearance of talc from the lung was impaired, or impaired to a greater extent, in mice exposed to the higher concentration.

Analysis of bronchoalveolar lavage fluid has been used in human medicine for diagnosing the type or stage of various forms of interstitial lung disease and

more recently as a rapid *in vivo* method of evaluating lung injury in toxicologic studies (Henderson *et al.*, 1985). Bronchoalveolar lavage was performed on rats and mice exposed to talc to evaluate its usefulness in chronic toxicology studies. Qualitatively similar changes in lavage fluid enzymes and cytology were observed in both species. Increases in neutrophils and total protein in lavage fluid are sensitive indicators of inflammation, and the increases in these parameters in rats and mice exposed to talc are consistent with the inflammation observed histologically in the lungs. Increases in cytoplasmic (lactate dehydrogenase and glutathione reductase) and lysosomal (β -glucuronidase) enzymes, which are indicative of cellular injury, were also observed in both species. Whether lactate dehydrogenase and glutathione reductase were derived from parenchymal cells or inflammatory cells is unknown. The increase in glutathione reductase suggests that cellular injury may have involved an oxidative process involving free radicals produced during phagocytosis.

The phagocytic ability of alveolar macrophages recovered from lavage fluid was not impaired in rats exposed to talc for 24 months, as indicated by the lack of a significant difference in the number of viable macrophages and the percentage of cells phagocytizing sheep erythrocytes in exposed and control rats. In contrast, both the viability and the phagocytic ability of alveolar macrophages from exposed mice were significantly lower than those of macrophages from controls. The percent of macrophages containing phagocytized erythrocytes decreased as aerosol concentration and exposure duration increased. Since alveolar macrophages play a major role in the clearance of particles from the lung, the decreased viability and phagocytic ability of these cells may explain the disproportionately greater lung talc burden in mice exposed to 18 mg/m³ than in mice exposed to 6 mg/m³, and the difference in talc lung burdens between exposed rats and mice.

Due to limitations in chamber size and the number of animals that could be exposed, the numbers of animals utilized in the lung biochemistry studies were generally small. Therefore, some of the apparent inconsistencies in the results of these studies can be attributed to the small sample sizes as well as the biologic variation in pulmonary response among individuals. Despite these limitations, increases in lavage fluid collagenous peptides and total lung collagen were observed in both rats and mice exposed to 18 mg/m³ talc. In rats, these

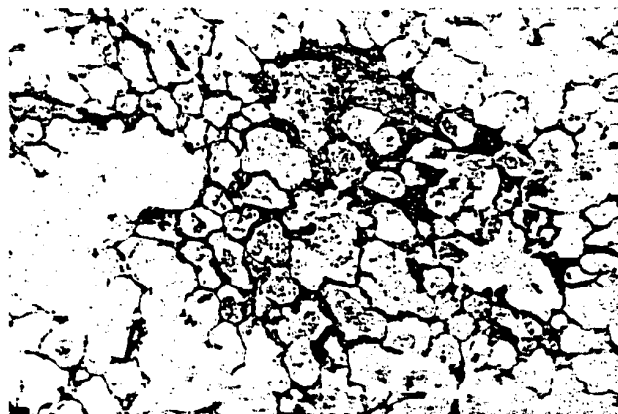


PLATE 1

Mild focal inflammation with thickening of the alveolar septa and distortion of the alveoli in lung of a male F344/N rat exposed to 18 mg/m³ talc at the 18-month interim evaluation of the lifetime inhalation study. H&E, 25X

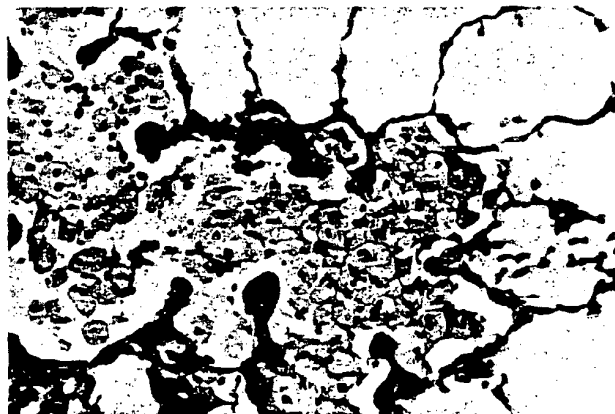


PLATE 2

Lung of a male F344/N rat exposed to 18 mg/m³ talc at the 18-month interim evaluation of the lifetime inhalation study. Note the accumulation of alveolar macrophages with pale granular cytoplasm in the alveolar duct and slight thickening of the septal walls. H&E, 80X

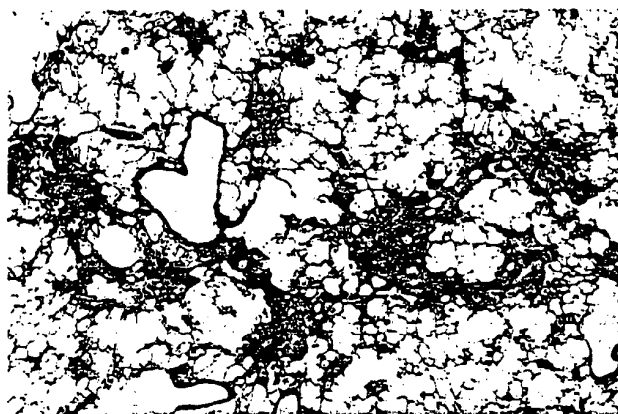


PLATE 3

Individual and confluent foci of interstitial fibrosis extend throughout the pulmonary parenchyma of a male F344/N rat exposed to 18 mg/m³ talc at the 24-month interim evaluation of the lifetime inhalation study. H&E, 6.6X

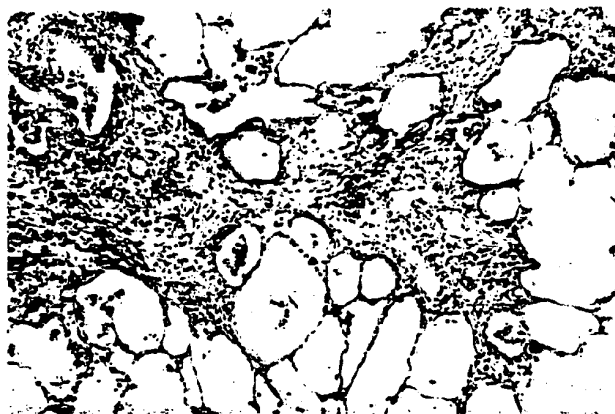


PLATE 4

Higher magnification of Plate 3 showing accumulation of fibrous tissue and interspersed inflammatory cells which obliterate the alveoli. H&E, 33X



PLATE 5

Squamous metaplasia and hyperplasia of the alveolar epithelium adjacent to an area of chronic inflammation and interstitial fibrosis in the lung of a male F344/N rat exposed to 18 mg/m³ talc in the lifetime inhalation study. H&E, 40X



PLATE 6

Alveolar/bronchiolar carcinoma in a male F344/N rat exposed to 18 mg/m³ talc in the lifetime inhalation study. Note the large mass obliterating the pulmonary parenchyma. H&E, 2.5X



PLATE 7

Higher magnification of the alveolar/bronchiolar carcinoma in Plate 6 showing neoplastic epithelium arranged in irregular papillary formations. H&E, 50X

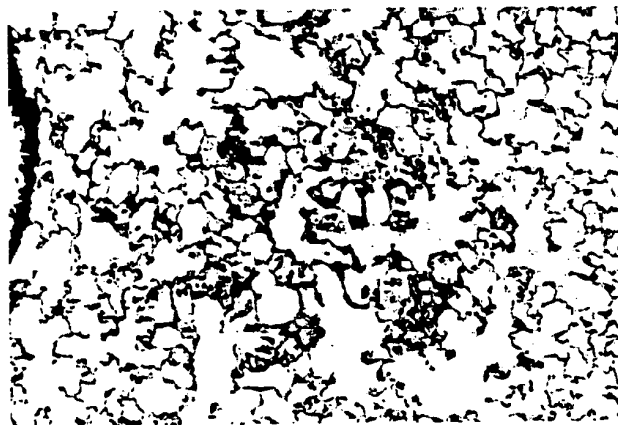


PLATE 8

Minimal focal accumulation of alveolar macrophages in the lung of a male B6C3F₁ mouse exposed to 18 mg/m³ talc at the 12-month interim evaluation of the 2-year inhalation study. H&E, 50X

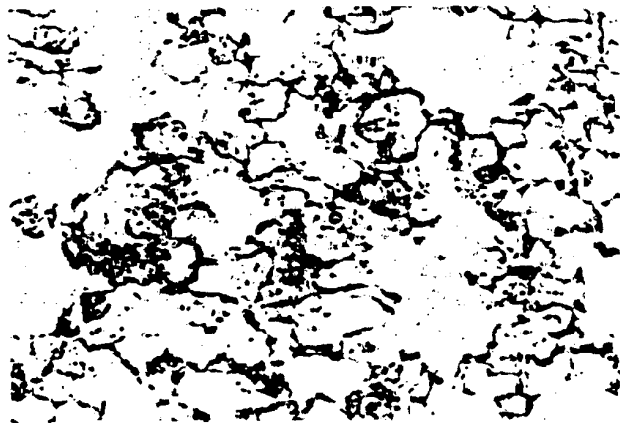


PLATE 9

Mild chronic active inflammation with slight thickening of the alveolar septa in the lung of a female B6C3F₁ mouse exposed to 18 mg/m³ talc in the 2-year inhalation study. H&E, 50X

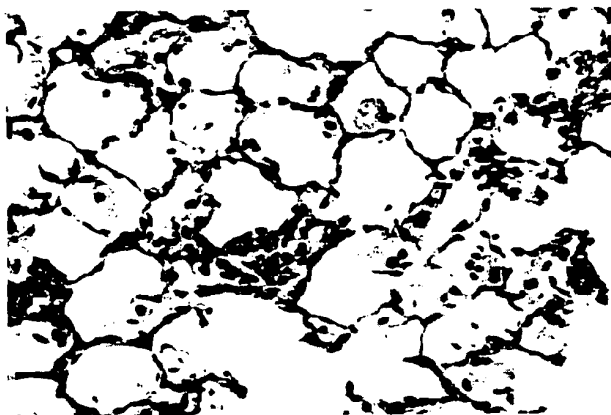


PLATE 10

Alveolar macrophages in alveoli and mononuclear cells in the interstitium of the lung of a male B6C3F₁ mouse exposed to 18 mg/m³ talc in the 2-year inhalation study. H&E, 100X

Discussion and Conclusions

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changes were also accompanied by increases in noncollagenous protein synthesis (total ^{14}C -proline incorporated into lung tissue minus that incorporated into collagen), and, in females only, an increase in collagen production (% of total ^{14}C -proline incorporated into collagen). Some parameters were also significantly increased in rats exposed to 6 mg/m^3 talc. While these results are consistent with the fibrosis observed histologically in rats, fibrosis was not seen histologically in mice.

Talc exposure was associated with a dose- and time-related impairment of respiratory functions in male and female rats. Although only slight trends were observed at 6 months in rats exposed to 18 mg/m^3 talc, functional alterations in rats at the high concentration were clearly evident after 11 months. In rats exposed to 6 mg/m^3 , decrements in respiratory function were observed in males at 11 months and in males and females at 18 months. The functional impairment was characterized by reduced lung volumes and reduced dynamic and/or quasistatic lung compliance, indicating an increase in elastic recoil (increased lung stiffness). Further, reduced gas exchange efficiency and nonuniform intrapulmonary gas distribution were also observed. These changes are consistent with the multifocal fibrosis and inflammation that was centered around the centriacinar region of the lung.

Deposition of talc in the lungs of rats and mice produced an inflammatory response characterized primarily by the accumulation of alveolar macrophages and, to a lesser extent, neutrophils and monocytes within alveolar lumens. Smaller numbers of lymphocytes and plasma cells were also observed in the interstitial tissue surrounding airways, blood vessels, and alveolar septa. The lesions developed at the junction of the alveolar ducts and terminal bronchioles where particles of the size range used are known to be deposited (Brody and Roe, 1983). Although the inflammatory response was basically similar in rats and mice, there were important species differences. The lesions in rats were generally more extensive and more severe than those in mice at similar exposure concentrations. In rats, foreign body giant cells were occasionally seen and some of the alveolar macrophages developed the morphological characteristics of epithelioid macrophages. More importantly, the inflammatory lesions in rats were accompanied by interstitial fibrosis, hyperplasia of alveolar epithelial type II cells, and, infrequently, squamous metaplasia of the alveolar epithelium.

The differences in pulmonary response cannot be attributed to differences in lung talc burden, since fibrosis and alveolar epithelial hyperplasia were seen in rats exposed to 6 mg/m^3 , which had lung talc burdens less than that of mice exposed to 18 mg/m^3 . Saffiotti and Stinson (1988) have reported similar differences in pulmonary response between rats and mice following intratracheal instillation of silica. These authors found that silica-induced alveolar epithelial hyperplasia in mice was transient, returning to normal within several months, while that in rats was generally more severe and persisted until the end of the study. Since inhalation studies using both rats and mice are seldom performed, it is uncertain if this species difference might exist for other particulate substances.

The difference in pulmonary response between rats and mice may be related, in part, to species differences in reactivity of the alveolar macrophage following phagocytosis of the talc particles. As the principal phagocytic cell of the lung, the alveolar macrophage is believed to play a major role in the inflammatory and fibrogenic reactions to inhaled particles (Brain, 1980; Brody, 1991). Much of the early work in this area centered on the differential cytotoxicity of phagocytized particles, particularly the various crystalline forms of asbestos and silica, to alveolar macrophages and the subsequent release of lysosomal enzymes which have proteolytic, elastolytic, and inflammatory properties (Brody and Davis, 1982; Nathan, 1987). More recently, alveolar macrophages have been shown to produce arachidonic acid metabolites (Kouzan *et al.*, 1985) and various cytokines that regulate cell proliferation, differentiation, and extracellular matrix production (Kelley, 1990). Of particular interest, rat alveolar macrophages exposed to iron spheres and asbestos fibers have been shown to produce increased amounts of a homologue of platelet-derived growth factor (Bonner *et al.*, 1989, 1990), the most potent mitogen known for mesenchymal cells, and TGF- β , a potent inhibitor of mesenchymal cell proliferation and stimulator of matrix production (Kalter *et al.*, 1989). Little is known about the putative role of PDGF and TGF- β and other macrophage-derived products in the pathogenesis of lung disease, but they are likely to be important mediators of many cellular events.

The lesions in the lungs of rats exposed to aerosols of talc are very similar, qualitatively, to those reported to occur following long-term (approximately 2 years) exposure to other inorganic,

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non-fibrous, particulate substances including titanium dioxide (Lee *et al.*, 1985), chromium dioxide (Lee *et al.*, 1988), antimony trioxide and antimony ore concentrate (predominantly antimony trisulfide) (Groth *et al.*, 1986), and volcanic ash (Wehner *et al.*, 1986). Aerosols of each of these particulate substances were reported to elicit pulmonary inflammation, characterized primarily by the accumulation of alveolar macrophages, hyperplasia and squamous metaplasia of the alveolar epithelium, and fibrosis. Since the various components of the pulmonary response were not quantified in these studies, there may be quantitative differences in the degree of inflammation, fibrosis, and cellular degenerative hyperplastic and metaplastic changes to these particulate substances.

The lesions in rats exposed to talc are also similar to those observed in rats exposed to silica, but with important differences. Silica generally produces an inflammatory response that is more pronounced and persistent than the response to the relatively more inert particles like titanium dioxide and talc (Saffiotti and Stinson, 1988; Driscoll *et al.*, 1990). Further, while only occasional multinucleated cells and epithelioid macrophages were seen in the cellular response to talc, rats exposed to silica develop discrete nodular aggregates of epithelioid macrophages with some multinucleated cells more typical of granulomatous inflammation.

The quantitative and qualitative differences in pulmonary toxicity to inhaled particles are likely related to their size, structure (amorphous, crystalline, and/or fibrous), surface chemistry, solubility (or durability), chemistry of soluble components, cytotoxicity, and other factors. While much of the research in this area has focused on asbestos (as well as other fibers) and silica, the same principles are likely to explain the differences in biological activity of other particulate substances. Although a complete discussion of these factors is beyond the scope of this report, some of the evidence is presented here.

A number of studies of the various forms of silicon dioxide have shown that amorphous silica produces the mildest, slowest developing pulmonary changes followed, in ascending order, by quartz, cristobalite and tridymite (Allison, 1977; Hemenway *et al.*, 1986). Amorphous silica generally lacks a detectable crystalline X-ray diffraction pattern, while, of the crystalline forms, quartz has a less ordered symmetry than cristobalite and tridymite. Moreover, stishovite, which lacks the tetrahedral structure of other forms

of silica, also lacks the fibrogenicity and cytotoxicity of the other forms (Brieger and Gross, 1967).

In general, the ability of various forms of silica to elicit pulmonary fibrosis parallels their cytotoxicity *in vitro* to alveolar macrophages (Reiser and Last, 1979). Further, there is a correlation between cytotoxicity and hemolytic activity *in vitro* (Allison, 1977). The biochemical basis of macrophage cytotoxicity and hemolytic activity is not fully understood, but the surface of crystalline silica presents highly reactive hydroxyl groups of silicic acid residues (silanol) that act as proton-donors and may combine with constituents of cellular membranes (Langer and Nolan, 1986). Kaolinite (aluminum silicate), mica (potassium aluminum silicate), and talc (magnesium silicate) are also hemolytic *in vitro* (Narang *et al.*, 1977). Dissolution of silicic acid residues from kaolinite, mica, and talc reduces the toxicity of these particulates, supporting the hypothesis that the reactive hydroxyl groups play an important role in cytotoxicity and hemolytic activity.

Following phagocytosis of silica (Allison, 1977) or kaolinite (Brody and Davis, 1982) particles by alveolar macrophages, hydrolytic enzymes are released from secondary lysosomes apparently as a result of the interaction of the particles with the lysosomal membrane. While the release of lysosomal enzymes into the cytoplasm may be directly responsible for cell death, it is less clear to what extent lysosomal enzymes released from the cells contribute to the other pulmonary lesions. Certainly, the ability to kill alveolar macrophages (cytotoxicity) is likely to inhibit or delay removal of the particles from the lung, increase the lung burden, and allow other biological effects to occur.

As already mentioned, macrophages secrete a large number of molecules with a wide range of biological functions including polypeptide hormones or cytokines, complement components, coagulation factors, arachidonic acid and its metabolites, bioactive lipids (prostaglandins and leukotrienes), binding proteins, enzyme inhibitors, extracellular matrix or cell adhesion proteins, and others (for review see Nathan, 1987). Some, or perhaps many, of the apparent differences in the pulmonary response of rats to the various particulate substances may be related to the extent to which they cause cytotoxicity and nonspecific release of lysosomal enzymes or cause macrophages to secrete specific effector substances like the cytokines and inflammatory mediators.

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Discussion and Conclusions

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Exposure of female rats to 18 mg/m³ talc was associated with increased incidences of benign and malignant pulmonary neoplasms (alveolar/bronchiolar adenoma: 1/50, 0/48, 9/50; alveolar/bronchiolar carcinoma: 0/50, 0/48, 5/50; squamous cell carcinoma: 0/50, 0/48, 1/50). The overall incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in female rats of the high-concentration group was significantly ($P \leq 0.001$) greater than that of controls (1/50, 0/48, 13/50). The incidence of pulmonary neoplasms in female rats exposed to 18 mg/m³ also greatly exceeds that of control females (8/529, 1.5%) in the NTP lifetime studies reported by Solleveld *et al.* (1984). While comparison with the historical controls from NTP lifetime studies has some limitations (e.g., the studies were conducted about a decade ago and are not contemporary), such a comparison provides some perspective. The increased incidence of pulmonary neoplasms in the 18 mg/m³ female rats was considered clear evidence of carcinogenic activity based on a) the strength of the statistical evidence ($P \leq 0.001$), b) the increase in malignant as well as benign neoplasms, and c) comparison with lifetime historical controls.

In contrast to female rats, there was no increase in the incidence of pulmonary neoplasms in male rats or in male or female mice exposed to talc aerosols. While precise comparisons between studies of talc and other particulate substances cannot be made because of differences in route of administration (intratracheal versus inhalation), strain of rat used, and exposure duration, such comparison provides some perspective (Table 12). The predilection of female rats over male rats for developing pulmonary neoplasms has also been observed in 2-year inhalation studies of titanium dioxide (Lee *et al.*, 1985), chromium dioxide (Lee *et al.*, 1988), antimony trioxide and antimony ore concentrate (predominantly antimony trisulfide) (Groth *et al.*, 1986), volcanic ash (Wehner *et al.*, 1986), and quartz (Dagle *et al.*, 1986). Chromium dioxide, volcanic ash, antimony trioxide, and antimony ore concentrate induced pulmonary neoplasms only in female rats, whereas titanium dioxide and quartz induced pulmonary neoplasms in males and females with a preponderance of neoplasms in females.

The morphological types of neoplasms induced by the particulates in the studies cited above also vary somewhat. The neoplasms in female rats exposed to talc were primarily alveolar/bronchiolar adenomas and carcinomas, although one squamous cell

carcinoma also occurred. In female rats exposed to antimony trioxide or antimony ore concentrate (Groth *et al.*, 1986), there were similar numbers of alveolar/bronchiolar neoplasms and squamous cell carcinomas (Table 12). Further, several scirrhous carcinomas were seen in antimony exposed rats. In female rats exposed to titanium dioxide (Lee *et al.*, 1985), the incidences of alveolar/bronchiolar neoplasms and squamous cell carcinoma were also similar, whereas all but one of the neoplasms in males were alveolar/bronchiolar neoplasms. In contrast, nearly all the pulmonary neoplasms induced by quartz (Dagle *et al.*, 1986), volcanic ash (Wehner *et al.*, 1986) or chromium dioxide (Lee *et al.*, 1988) were squamous cell (epidermoid) carcinomas.

The pathogenesis of pulmonary neoplasms induced by the relatively insoluble particulate substances, such as talc, is currently unknown. Although a genotoxic mechanism cannot be ruled out, there are several facts and lines of evidence to suggest that a direct effect of the particulate on the target cell genome is not involved. First, the insoluble nature of these particulates makes it unlikely that any chemical constituents will reach sufficient concentration to affect the target cells within the relatively short period between the time they are deposited on the alveolar surface and the time they are phagocytized. Further, although occasional alveolar epithelial cells have been observed to contain particles following intratracheal or inhalation exposure (Sorokin and Brian, 1975; Lee *et al.*, 1979), the vast majority of particles are rapidly phagocytized by alveolar macrophages, some within minutes of deposition in the lung (Lauweryns and Baert, 1974). It is also clear that physical characteristics (crystalline structure, fiber dimension) and surface chemistry (presence of reactive groups on the particle surface), rather than soluble chemical components, are principle determinants of tissue reaction, and perhaps for carcinogenicity. The carcinogenicity of many fibrous materials (fiberglass, attapulgite, silicon carbide, mineral wool, and potassium titanate) decreases as fiber diameter exceeds 2.5 μm and as fiber length decreases below 10 μm (Stanton and Wrench, 1972; Stanton *et al.*, 1977).

A potential mechanism for the development of pulmonary neoplasms associated with insoluble particulate substances is that the prolonged stimulus for cell replication, due not only to cell injury but to the release of mitogenic growth factors from alveolar macrophages, provides a favorable

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TABLE 12
Results of Selected Whole Body Inhalation Carcinogenicity Studies of Particulate Materials

Compound and Dose	Study Duration	Species	Effects ^a
Talc at 0, 6, or 18 mg/m ³ (NTP, 1992)	Male: 113 weeks Female: 122 weeks	F344/N rats	Females: alveolar/bronchiolar adenoma (1/50, 0/48, 9/50); alveolar/bronchiolar carcinoma (0/50, 0/48, 5/50); squamous cell carcinoma (0/50, 0/48, 1/50)
Titanium dioxide at 0, 10, 50, or 250 mg/m ³ (Lee <i>et al.</i> , 1985)	104 weeks	CD rats	Females: alveolar/bronchiolar adenoma (0/77, 0/75, 0/74, 13/74); squamous cell carcinoma (0/77, 0/75, 0/74, 13/74)
Titanium tetrachloride at 0, 0.1, 1.0, or 10 mg/m ³ (Lee <i>et al.</i> , 1986)	104 weeks	Crl:CD rats	Females: squamous cell carcinoma (0/77, 0/75, 0/79, 3/75); Males: squamous cell carcinoma (0/79, 0/77, 0/78, 2/75)
Chromium dioxide at 0, 0.5, 0.5 ^b , or 25 mg/m ³ (Lee <i>et al.</i> , 1988)	104 weeks	Sprague-Dawley rats	Females: squamous cell carcinoma (0/106, 0/103, 0/108, 2/108); keratin cyst (0/106, 0/103, 0/108, 6/108)
Antimony trioxide at 0 or 45 mg/m ³ (Groth <i>et al.</i> , 1986)	73 weeks	Wistar rats	Females: alveolar/bronchiolar neoplasms (0/90, 11/90); squamous cell carcinoma (0/90, 9/90); scirrhous carcinoma (0/90, 5/90)
Antimony trisulfide at 0 or 40 mg/m ³ (Groth <i>et al.</i> , 1986)	72 weeks	Wistar rats	Females: alveolar/bronchiolar neoplasms (0/90, 6/90); squamous cell carcinoma (0/90, 9/90); scirrhous carcinoma (0/90, 4/90)
Volcanic ash at 0, 5, or 50 mg/m ³ (Wehner <i>et al.</i> , 1986)	up to 104 weeks	F344 rats	Females: several ^c squamous cell carcinomas in the 50 mg/m ³ group. Male: one squamous cell carcinoma in the 50 mg/m ³ group.
Quartz at 0 or 50 mg/m ³ (Wehner <i>et al.</i> , 1986)	up to 104 weeks	F344 rats	Females: moderate ^c numbers of squamous cell carcinomas in the 50 mg/m ³ group. Males: one squamous cell carcinoma in the 50 mg/m ³ group.

^a Tumor incidences are given as the number of animals with tumor per number of animals examined. The incidences are given in the order of increasing exposure concentration.

^b This dose represents unstabilized chromium dioxide; the other doses represent stabilized chromium dioxide.

^c Precise numbers not available in journal article.

environment for the promotion and progression of spontaneously initiated cells. The interim evaluations in the NTP talc study clearly demonstrate a progressive impairment of homeostatic growth regulation in the areas of chronic inflammation and fibrosis associated with talc deposition in rats. Hyperplasia of the alveolar epithelium was evident at 6 months and became more extensive and severe with duration of exposure. Not only were there increased numbers of cells (hyperplasia), but some cells assumed morphologic features atypical of regenerating or differentiated type II cells (epithelial dysplasia). The altered or dysplastic epithelium was particularly evident in areas of

fibrosis. The squamous metaplasia observed in female rats also represents altered differentiation of populations of alveolar epithelial cells and is notable in light of the development of squamous cysts and squamous cell carcinomas.

The lack of a carcinogenic effect in male rats or in mice exposed to talc aerosols does not negate the possibility of a mechanism as described above. First, the difference between male and female rats may be one of magnitude rather than an absolute difference in effect. The influence of the length of exposure on the development of these late appearing lung neoplasms cannot be discounted; the length of

Discussion and Conclusions

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exposure was 113 weeks for males and 122 weeks for females. Further, the promotion and progression of neoplasia involve a complex series of molecular events that are likely to differ qualitatively or quantitatively in males and females. Clearly, there are sex differences in the incidence of spontaneous and chemically induced neoplasms. As for mice exposed to talc, there was no histologic evidence of impaired growth regulation or fibrosis, consistent with the mechanism proposed above.

Pheochromocytomas (benign, malignant, or complex) of the adrenal medulla occurred with significant positive trends in both male and female rats exposed to talc (males: 26/49, 32/48, 37/47; females: 13/48, 14/47, 23/49). Further, the numbers of male and female rats with bilateral pheochromocytomas were also increased in the exposed groups. The overall incidences of this neoplasm in the 18 mg/m³ exposure groups were significantly greater than those of the controls. Comparison with historical controls of NTP lifetime studies is not considered relevant, since there has been a pronounced increase in the spontaneous occurrence of pheochromocytomas in male rats in studies conducted by the NTP over the last 10 years (Rao *et al.*, 1990).

In contrast to the pheochromocytomas, the incidences of adrenal medulla hyperplasia in exposed male rats were lower than in controls, and the incidences were similar in all female groups. Because of the small size of the adrenal medulla, pheochromocytomas tend to obscure much or all of the remaining tissue. Therefore, the lower incidences of hyperplasia in groups of exposed males can be attributed, in part, to the larger number of pheochromocytomas.

While the increased incidences of pheochromocytomas in male rats were exposure related, it was believed to represent some, rather than clear, evidence of carcinogenic activity because a) the increase was associated primarily with benign neoplasms and b) there was no supporting increase in the incidence of hyperplasia. The increased incidence of pheochromocytomas in female rats was also exposure related.

Although the strength of the statistical association indicates that the pheochromocytomas are exposure related, a plausible mechanism for their increased occurrence in rats exposed to talc aerosols is not readily apparent. Since talc is relatively insoluble, it is extremely unlikely that any soluble components could have reached concentrations high enough in

the blood to affect the adrenal medulla cells. Although purely speculative, there are two general hypotheses that might be considered. First, the increased incidence of adrenal pheochromocytomas may be a nonspecific effect of stress as a result of the chronic pulmonary inflammation. The body is known to respond to an exogenous challenge such as injury, inflammation, or infection by a set of distinct physiologic, metabolic, and endocrine changes including increases in serum adrenocorticotrophic hormone and cortisone levels, growth hormone, and catecholamine synthesis. Further, the adrenal medulla, as a modified sympathetic ganglia, reacts to neural as well as hormonal stimuli in the secretion of catecholamines. While prolonged stimulus of secretion is coupled with cellular hypertrophy and hyperplasia (cell proliferation) in many endocrine tissues, it is unknown if this occurs in the adrenal medulla. Moreover, if prolonged stress were to increase the rate of occurrence or growth of medullary proliferative lesions, similar exposure-related increases in pheochromocytoma incidence might be expected in other chronic toxicity and carcinogenicity studies. This has not generally been the case. Exposure-related increased incidences of pheochromocytoma were either not observed or not reported in the 2-year inhalation studies of other particulate substances reported above.

A second hypothesis to consider is that cytokines (growth factors), released from macrophages or other cells in the lung, might be responsible for increasing the rate of growth of pheochromocytomas. Although alveolar macrophages have been shown to secrete a number of cytokines known to stimulate proliferation of a variety of cell types, cytokines are generally believed to affect cells only in close proximity within the same organ. However, it has recently been shown that measurable levels of hepatocyte growth factor are present in the plasma after two-thirds hepatectomy (Lindroos *et al.*, 1992). Thus, some cytokines or growth factors may have wider effects than currently known.

CONCLUSIONS

Under the conditions of these inhalation studies, there was *some evidence of carcinogenic activity** of talc in male F344/N rats based on an increased incidence of benign and malignant pheochromocytomas of the adrenal gland. There was *clear evidence of carcinogenic activity* of talc in female F344/N rats based on increased incidences of alveolar/bronchiolar adenomas and carcinomas of

the lung and benign and malignant pheochromocytomas of the adrenal gland. There was *no evidence of carcinogenic activity* of talc in male or female B6C3F₁ mice exposed to 6 or 18 mg/m³.

The principal toxic lesions associated with inhalation exposure to talc in rats included chronic granulomatous inflammation, alveolar epithelial hyperplasia, squamous metaplasia and squamous cysts, and

interstitial fibrosis of the lung. These lesions were accompanied by impaired pulmonary function characterized primarily by reduced lung volumes, reduced dynamic and/or quasistatic lung compliance, reduced gas exchange efficiency, and nonuniform intrapulmonary gas distribution. In mice, inhalation exposure to talc produced chronic inflammation of the lung with the accumulation of alveolar macrophages.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9.

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A-1

APPENDIX A SUMMARY OF LESIONS IN MALE RATS IN THE LIFETIME INHALATION STUDY OF TALC

TABLE A1	Summary of the Incidence of Neoplasms in Male Rats in the Lifetime Inhalation Study of Talc	A-2
TABLE A2	Individual Animal Tumor Pathology of Male Rats in the Lifetime Inhalation Study of Talc	A-6
TABLE A3	Statistical Analysis of Primary Neoplasms in Male Rats in the Lifetime Inhalation Study of Talc	A-24
TABLE A4	Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Lifetime Inhalation Study of Talc	A-28

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TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the Lifetime Inhalation Study of Talc^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Moribund	23	19	20
Natural deaths	18	17	14
Survivors			
Died last week of study	1	2	3
Terminal sacrifice	8	12	13
Animals examined microscopically	49	50	50
Alimentary System			
Intestine large, cecum	(42)	(38)	(37)
Intestine large, colon	(43)	(43)	(46)
Intestine small, duodenum	(48)	(44)	(46)
Intestine small, ileum	(39)	(34)	(35)
Intestine small, jejunum	(40)	(38)	(40)
Liver	(49)	(50)	(48)
Neoplastic nodule			1 (2%)
Neoplastic nodule, multiple	2 (4%)	1 (2%)	3 (6%)
Osteosarcoma, metastatic, multiple, bone	1 (2%)		
Hepatocyte, adenoma		1 (2%)	
Mesentery	(2)		(1)
Pancreas	(48)	(46)	(47)
Salivary glands	(49)	(50)	(50)
Fibroma		1 (2%)	
Stomach, forestomach	(49)	(47)	(47)
Fibrosarcoma			1 (2%)
Stomach, glandular	(49)	(47)	(47)
Fibrosarcoma			1 (2%)
Cardiovascular System			
Heart	(49)	(50)	(50)
Endocrine System			
Adrenal gland, cortex	(49)	(49)	(48)
Adrenal gland, medulla	(49)	(48)	(47)
Osteosarcoma, metastatic, uncertain primary site			1 (2%)
Pheochromocytoma malignant	2 (4%)	3 (6%)	6 (13%)
Pheochromocytoma complex		2 (4%)	1 (2%)
Pheochromocytoma benign	13 (27%)	9 (19%)	20 (43%)
Bilateral, pheochromocytoma malignant	1 (2%)		1 (2%)
Bilateral, pheochromocytoma benign	12 (24%)	21 (44%)	16 (34%)
Islets, pancreatic	(47)	(41)	(43)
Adenoma	1 (2%)		2 (5%)
Carcinoma	1 (2%)		
Parathyroid gland	(45)	(45)	(46)
Adenoma		1 (2%)	
Pituitary gland	(47)	(50)	(49)
Pars distalis, adenoma	12 (26%)	11 (22%)	10 (20%)
Pars distalis, carcinoma		1 (2%)	
Pars intermedia, adenoma			2 (4%)

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Lesions in Male Rats

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TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the Lifetime Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Endocrine System (continued)			
Thyroid gland	(45)	(46)	(46)
C-cell, adenoma	3 (7%)	4 (9%)	3 (7%)
C-cell, carcinoma		1 (2%)	
Follicular cell, adenoma			1 (2%)
General Body System			
Tissue NOS	(1)	(1)	
Pheochromocytoma malignant, metastatic, adrenal gland		1 (100%)	
Genital System			
Epididymis	(49)	(50)	(49)
Preputial gland	(48)	(49)	(48)
Adenoma	1 (2%)	1 (2%)	1 (2%)
Carcinoma	1 (2%)	6 (12%)	1 (2%)
Prostate	(49)	(45)	(48)
Seminal vesicle	(49)	(48)	(47)
Testes	(49)	(50)	(50)
Bilateral, interstitial cell, adenoma	18 (37%)	24 (48%)	24 (48%)
Interstitial cell, adenoma	13 (27%)	15 (30%)	12 (24%)
Hematopoietic System			
Bone marrow	(48)	(48)	(47)
Lymph node	(49)	(50)	(50)
Lymph node, bronchial	(41)	(48)	(49)
Lymph node, mandibular	(46)	(48)	(47)
Lymph node, mediastinal	(48)	(49)	(47)
Lymph node, mesenteric	(49)	(48)	(47)
Spleen	(49)	(50)	(48)
Fibrosarcoma	1 (2%)		
Fibrous histiocytoma		1 (2%)	
Osteosarcoma, metastatic, bone	1 (2%)		
Thymus	(48)	(40)	(43)
Thymoma malignant	1 (2%)		
Integumentary System			
Mammary gland	(45)	(48)	(50)
Adenocarcinoma	1 (2%)		
Skin	(48)	(50)	(50)
Basosquamous tumor malignant			1 (2%)
Fibroma		2 (4%)	
Fibrous histiocytoma			1 (2%)
Keratoacanthoma		2 (4%)	2 (4%)
Neurofibroma		1 (2%)	
Squamous cell carcinoma		1 (2%)	
Subcutaneous tissue, fibroma		1 (2%)	
Subcutaneous tissue, fibrosarcoma		1 (2%)	
Subcutaneous tissue, schwannoma malignant	1 (2%)		

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TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the Lifetime Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Musculoskeletal System			
Bone	(49)	(50)	(50)
Pelvis, osteosarcoma		1 (2%)	
Scapula, osteosarcoma	1 (2%)		1 (2%)
Vertebra, osteosarcoma			
Skeletal muscle	(1)		
Nervous System			
Brain	(49)	(50)	(50)
Astrocytoma malignant	1 (2%)		
Respiratory System			
Lung	(49)	(50)	(50)
Alveolar/bronchiolar adenoma		1 (2%)	1 (2%)
Alveolar/bronchiolar carcinoma, multiple			1 (2%)
Fibrosarcoma, metastatic, salivary glands	1 (2%)		
Osteosarcoma, metastatic		1 (2%)	
Osteosarcoma, metastatic, uncertain primary site			1 (2%)
Osteosarcoma, metastatic, multiple, bone	1 (2%)		
Nose	(49)	(48)	(47)
Chondroma	1 (2%)		
Sarcoma		1 (2%)	
Special Senses System			
None			
Urinary System			
Kidney	(49)	(49)	(48)
Renal tubule, carcinoma	2 (4%)		
Urinary bladder	(49)	(48)	(47)
Papilloma	1 (2%)		
Systemic Lesions			
Multiple organs^b	(49)	(50)	(50)
Leukemia mononuclear	24 (49%)	21 (42%)	23 (46%)
Lymphoma malignant lymphocytic	1 (2%)		
Mesothelioma benign	1 (2%)		
Mesothelioma malignant			1 (2%)

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Lesions in Male Rats

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TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the Lifetime Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Tumor Summary			
Total animals with primary neoplasms ^c	48	49	50
Total primary neoplasms	116	135	137
Total animals with benign neoplasms	42	45	45
Total benign neoplasms	78	96	98
Total animals with malignant neoplasms	34	33	33
Total malignant neoplasms	38	39	39
Total animals with metastatic neoplasms	2	2	1
Total metastatic neoplasms	4	2	2
Total animals with malignant neoplasms, uncertain primary site			1

^a Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

^b Number of animals with any tissue examined microscopically

^c Primary tumors: all tumors except metastatic tumors

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TABLE A2
Individual Animal Tumor Pathology of Male Rats in the Lifetime Inhalation Study of Talc: 0 mg/m³

	3	3	4	5	5	5	5	5	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7
Number of Days on Study	3	6	2	5	6	8	9	9	2	2	3	3	5	5	7	8	8	9	0	0	0	2	3
	4	0	9	1	8	6	0	3	2	8	1	5	0	6	0	2	2	8	0	0	4	9	4
	3	3	3	3	4	2	2	3	3	4	3	3	3	4	3	3	3	3	3	3	3	2	4
Carcass ID Number	6	0	6	4	1	9	9	1	8	2	3	4	6	1	4	4	4	1	1	8	9	1	9
	1	0	8	0	3	4	5	8	7	0	9	2	3	8	5	3	8	7	6	5	0	3	6
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Alimentary System																							
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	M	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine large, rectum	M	+	+	+	+	+	+	+	M	+	+	+	A	M	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	A	+	+	+	A	+	A
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	A
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule, multiple																							
Osteosarcoma, metastatic, multiple, bone																							
Mesentery	+																						
Pancreas	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																							
Blood vessel				+												+							
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																							
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																							
Pheochromocytoma benign																							
Bilateral, pheochromocytoma malignant																							
Bilateral, pheochromocytoma benign																							
Islets, pancreatic	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																							
Carcinoma																							
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	I	+	+	+
Pars distalis, adenoma																							
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
C-cell, adenoma																							

+: Tissue examined microscopically
A: Autolysis precludes examination

M: Missing tissue
I: Insufficient tissue

X: Lesion present
Blank: Not examined

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TABLE A2
Individual Animal Tumor Pathology of Male Rats in the Lifetime Inhalation Study of Talc: 0 mg/m³ (continued)

Number of Days on Study	3	3	4	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7
	3	6	2	5	6	8	9	9	2	2	3	3	5	5	7	8	8	9	0	0	0	0	2	3
	4	0	9	1	8	6	0	3	2	8	1	5	0	6	0	2	2	8	0	0	4	9	4	9
Carcass ID Number	3	3	3	3	4	2	2	3	3	4	3	3	3	4	3	3	3	3	3	3	3	3	2	4
	6	0	6	4	1	9	9	1	8	2	3	4	6	1	4	4	4	1	1	8	9	1	9	1
	1	0	8	0	3	4	5	8	7	0	9	2	3	8	5	3	8	7	6	5	0	3	6	4
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
General Body System																								
Tissue NOS	+																							
Genital System																								
Epididymis	+																							
Preputial gland	+																							
Adenoma	X																							
Carcinoma	X																							
Prostate	+																							
Seminal vesicle	+																							
Testes	+																							
Bilateral, interstitial cell, adenoma	X																							
Interstitial cell, adenoma	X																							
Hematopoietic System																								
Bone marrow	+																							
Lymph node	+																							
Lymph node, bronchial	+																							
Lymph node, mandibular	+																							
Lymph node, mediastinal	+																							
Lymph node, mesenteric	+																							
Spleen	+																							
Fibrosarcoma	X																							
Osteosarcoma, metastatic, bone	X																							
Thymus	+																							
Thymoma malignant	X																							
Integumentary System																								
Mammary gland	M																							
Adenocarcinoma	M																							
Skin	M																							
Subcutaneous tissue, schwannoma malignant	X																							
Musculoskeletal System																								
Bone	+																							
Scapula, osteosarcoma	X																							
Skeletal muscle	+																							

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	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	8	8	8	8	8
Number of Days on Study	4	4	4	4	5	6	6	6	8	8	8	8	9	9	9	9	9	9	0	0	0	0	0
	0	1	5	6	7	9	1	4	6	2	4	5	6	7	0	5	9	9	9	0	0	0	0
Carcass ID Number	3	2	3	2	3	4	4	3	3	3	3	4	3	3	3	3	3	3	2	2	3	3	4
	6	9	6	9	1	1	1	9	4	2	8	8	1	2	9	3	2	6	7	8	9	9	2
	7	8	2	1	9	1	0	1	7	3	9	8	5	1	6	7	4	9	1	6	3	7	2
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Total Tissues/Tumors																							
General Body System																							
Tissue NOS																							
Genital System																							
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Adenoma																							
Carcinoma																							
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma	X	X			X	X	X	X	X	X					X	X		X	X	X	X		X
Interstitial cell, adenoma					X								X	X	X		X	X		X	X		
Hematopoietic System																							
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, bronchial	+	+	+	+	+	+	+	+	+	+	M	I	+	+	M	+	+	+	M	+	+	+	M
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Lymph node, mediastinal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma																							
Osteosarcoma, metastatic, bone																							
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Thymoma malignant																							
Integumentary System																							

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Talc, NTP TR 421

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the Lifetime Inhalation Study of Talc: 0 mg/m³ (continued)

Number of Days on Study	3 3 4 5 5 5 5 5 6 6 6 6 6 6 6 6 6 7 7 7 7 7 7
	3 6 2 5 6 8 9 9 2 2 3 3 5 5 7 8 8 9 0 0 0 0 2 3
	4 0 9 1 8 6 0 3 2 8 1 5 0 6 0 2 2 8 0 0 4 9 4 9
Carcass ID Number	3 3 3 3 4 2 2 3 3 4 3 3 3 4 3 3 3 3 3 3 3 2 4
	6 0 6 4 1 9 9 1 8 2 3 4 6 1 4 4 4 1 1 8 9 1 9 1
	1 0 8 0 3 4 5 8 7 0 9 2 3 8 5 3 8 7 6 5 0 3 6 4
	1 1
Nervous System	
Brain	+ +
Astrocytoma malignant	
Respiratory System	
Larynx	+ 1 +
Lung	+ +
Fibrosarcoma, metastatic, salivary glands	
Osteosarcoma, metastatic, multiple, bone	X
Nose	+ +
Chondroma	X
Trachea	+ +
Special Senses System	
Eye	+ +
Urinary System	
Kidney	+ +
Renal tubule, carcinoma	
Urinary bladder	+ +
Papilloma	
Systemic Lesions	
Multiple organs	+ +
Leukemia mononuclear	X X
Lymphoma malignant lymphocytic	
Mesothelioma benign	X

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TABLE A2
Individual Animal Tumor Pathology of Male Rats in the Lifetime Inhalation Study of Talc: 6 mg/m³

	1	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7		
Number of Days on Study	8	2	2	4	5	7	9	9	0	1	3	4	5	5	6	7	7	9	1	2	2	3	3	4	4		
	6	7	9	4	8	3	3	3	4	1	3	8	0	7	3	3	7	0	5	2	8	4	9	0	1		
Carcass ID Number	0	0	1	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	1		
	0	2	0	5	0	0	4	0	7	1	7	2	6	3	5	0	0	5	0	1	8	9	8	5	2		
	6	9	7	9	5	4	9	3	3	1	9	8	0	1	7	0	3	3	2	2	3	7	4	6	4		
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
<hr/>																											
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Intestine large	+	+	+	A	+	+	+	+	+	A	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+		
Intestine large, cecum	+	+	+	A	+	+	+	+	+	A	A	+	+	A	+	+	+	+	A	+	+	+	+	A	+		
Intestine large, colon	+	+	+	A	+	+	+	+	+	A	A	+	+	A	+	+	+	+	A	+	+	+	+	+	+		
Intestine large, rectum	+	+	+	A	+	M	+	+	M	+	A	+	+	A	+	+	+	+	A	+	+	+	+	+	+		
Intestine small	+	+	+	A	+	+	+	+	+	A	A	+	+	A	+	+	+	+	A	+	+	+	+	+	+		
Intestine small, duodenum	+	+	+	A	+	+	+	+	+	A	A	+	+	A	+	+	+	+	A	+	+	+	+	+	+		
Intestine small, ileum	+	+	+	A	+	+	+	+	+	A	A	+	+	A	+	+	+	+	A	+	+	+	+	A	A		
Intestine small, jejunum	+	+	+	A	+	+	+	+	+	A	A	+	+	A	+	+	+	+	A	+	+	+	+	+	+		
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Neoplastic nodule, multiple																											
Hepatocyte, adenoma																											
Pancreas	+	+	+	A	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	A	+	+	+	+	+		
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Fibroma																											
Stomach	+	+	+	A	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+		
Stomach, forestomach	+	+	+	A	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+		
Stomach, glandular	+	+	+	A	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+		
<hr/>																											
Cardiovascular System																											
Blood vessel																											
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
<hr/>																											
Endocrine System																											
Adrenal gland	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	A	+	+	I	+	+	+	+	+	+	+	+	+	+	+		
Pheochromocytoma malignant				X																							
Pheochromocytoma complex					X																						
Pheochromocytoma benign					X						X			X	X				X	X							
Bilateral, pheochromocytoma benign							X		X										X	X		X					
Islets, pancreatic	+	+	+	A	+	+	+	+	+	+	A	A	+	+	+	M	+	+	+	A	+	+	M	+	+		
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+		
Adenoma																			X								
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Pars distalis, adenoma						X										X				X		X					
Pars distalis, carcinoma																											
Thyroid gland	+	+	+	A	+	+	+	+	+	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	+		
C-cell, adenoma																											
C-cell, carcinoma																											

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Talc, NTP TR 421

TABLE A2

Individual Animal Tumor Pathology of Male Rats in the Lifetime Inhalation Study of Talc: 6 mg/m³ (continued)

	1	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7		
Number of Days on Study	8	2	2	4	5	7	9	9	0	1	3	4	5	5	6	7	7	9	1	2	2	3	3	4	4	
	6	7	9	4	8	3	3	3	4	1	3	8	0	7	3	3	7	0	5	2	8	4	9	0	1	
	0	0	1	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	1	
Carcass ID Number	0	2	0	5	0	0	4	0	7	1	7	2	6	3	5	0	0	5	0	1	8	9	8	5	2	
	6	9	7	9	5	4	9	3	3	1	9	8	0	1	7	0	3	3	2	2	3	7	4	6	4	
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
General Body System																										
Tissue NOS	+																									
Pheochromocytoma malignant, metastatic, adrenal gland	X																									
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																										
Carcinoma	X			X																			X	X		
Prostate	+	+	+	M	+	+	+	+	M	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	A
Seminal vesicle	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma									X		X	X				X	X	X	X			X	X		X	
Interstitial cell, adenoma				X	X				X					X		X				X						
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, bronchial	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	I	+	+	+	+
Lymph node, mediastinal	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma																										
Thymus	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	I	+	+	+
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma	X																									
Keratoacanthoma	X																									
Neurofibroma																										
Squamous cell carcinoma																										
Subcutaneous tissue, fibroma																										
Subcutaneous tissue, fibrosarcoma																										

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Talc, NTP TR 421

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the Lifetime Inhalation Study of Talc: 6 mg/m³ (continued)

Number of Days on Study	1 5 5 5 5 5 5 6 6 6 6 6 6 6 6 7 7 7 7 7 7
	8 2 2 4 5 7 9 9 0 1 3 4 5 5 6 7 9 1 2 2 3 3 4 4
	6 7 9 4 8 3 3 3 4 1 3 8 0 7 3 3 7 0 5 2 8 4 9 0 1
Carcass ID Number	0 0 1 0 1 0 0 1 0 0 0 1 0 0 0 1 0 0 1 0 0 0 0 0 1
	0 2 0 5 0 0 4 0 7 1 7 2 6 3 5 0 0 5 0 1 8 9 8 5 2
	6 9 7 9 5 4 9 3 3 1 9 8 0 1 7 0 3 3 2 2 3 7 4 6 4
	1 1
Musculoskeletal System	
Bone	+ +
Pelvis, osteosarcoma	X
Nervous System	
Brain	+ +
Respiratory System	
Larynx	+ +
Lung	+ +
Alveolar/bronchiolar adenoma	
Osteosarcoma, metastatic	X
Nose	+ + + + + + + + + A + + + + + + + + + + + +
Sarcoma	X
Trachea	+ +
Special Senses System	
Eye	+
Urinary System	
Kidney	+ + + A + + + + + + + + + + + + + + + + + +
Urinary bladder	+ + + + + + + + + A + + + + + + + + + + + +
Systemic Lesions	
Multiple organs	+ +
Leukemia mononuclear	X X X X X X X X X X X X X X X X X X

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	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	8	8	8	8	8	8	8	8	8	8	
Number of Days on Study	4	7	8	8	9	9	9	9	9	9	9	9	9	9	9	9	9	9	0	0	0	0	0	0	0	0	0	
	3	8	3	8	1	1	3	4	8	9	9	9	9	9	9	9	9	9	0	0	0	0	0	0	0	0	0	
Carcass ID Number	2	2	2	1	2	2	2	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	Total
	0	4	9	4	7	7	9	4	5	5	6	7	8	9	9	9	9	2	2	4	4	4	6	7	7	7	Tissues/	
	2	1	2	4	5	4	1	7	6	5	6	9	8	0	3	6	8	0	8	3	6	7	8	5	6	6	Tumors	
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
General Body System																												
None																												
Genital System																												
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Penis													+		+					+							3	
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48	
Adenoma															X												1	
Carcinoma																											1	
Prostate	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48	
Seminal vesicle	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47	
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Bilateral, interstitial cell, adenoma	X	X		X	X			X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	24	
Interstitial cell, adenoma					X						X	X		X			X										12	
Hematopoietic System																												
Bone marrow	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47	
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Lymph node, bronchial	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Lymph node, mandibular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47	
Lymph node, mediastinal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47	
Lymph node, mesenteric	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47	
Spleen	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48	
Thymus	A	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	43	
Integumentary System																												

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Talc, NTP TR 421

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the Lifetime Inhalation Study of Talc: 18 mg/m³ (continued)

Number of Days on Study	2	4	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	
	4	9	0	9	0	0	1	1	1	1	2	3	4	5	5	5	7	8	9	9	0	0	1	2	3
	8	2	0	4	7	9	4	5	5	7	8	4	5	1	1	3	6	3	7	8	1	5	9	2	7
Carcass ID Number	2	1	2	1	2	1	1	1	2	2	1	2	2	2	2	2	2	1	1	2	1	2	1	1	1
	1	4	0	7	4	7	7	4	2	6	9	1	5	0	2	6	7	2	7	5	2	5	5	5	7
	9	5	3	7	4	4	5	9	4	6	5	7	1	2	7	7	0	6	6	2	5	1	2	0	2
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+																								
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma, multiple																									
Osteosarcoma, metastatic, uncertain primary site																									X
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																									+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ureter																									+
Urethra																									
Urinary bladder	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X				X	X	X	X	X	X	X				X	X	X	X		X		X	X	X	
Mesothelioma malignant																									

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Talc, NTP TR 421

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the Lifetime Inhalation Study of Talc

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Adrenal Medulla: Benign Pheochromocytoma			
Overall rates ^a	25/49 (51%)	30/48 (63%)	36/47 (77%)
Adjusted rates ^b	87.6%	90.2%	100.0%
Terminal rates ^c	6/9 (67%)	11/14 (79%)	16/16 (100%)
First incidence (days)	429	558	614
Life table tests ^d	P=0.434	P=0.515N	P=0.499
Logistic regression tests ^d	P=0.007	P=0.213	P=0.009
Cochran-Armitage test ^d	P=0.007		
Fisher exact test ^e		P=0.175	P=0.008
Adrenal Medulla: Malignant Pheochromocytoma			
Overall rates	3/49 (6%)	3/48 (6%)	7/47 (15%)
Adjusted rates	17.2%	15.2%	31.5%
Terminal rates	1/9 (11%)	1/14 (7%)	3/16 (19%)
First incidence (days)	670	544	645
Life table tests	P=0.242	P=0.552N	P=0.376
Logistic regression tests	P=0.096	P=0.662	P=0.178
Cochran-Armitage test	P=0.083		
Fisher exact test		P=0.651	P=0.142
Adrenal Medulla: Benign, Malignant, or Complex Pheochromocytoma			
Overall rates	26/49 (53%)	32/48 (67%)	37/47 (79%)
Adjusted rates	91.7%	93.6%	100.0%
Terminal rates	7/9 (78%)	12/14 (86%)	16/16 (100%)
First incidence (days)	429	544	614
Life table tests	P=0.483	P=0.549N	P=0.539
Logistic regression tests	P=0.007	P=0.147	P=0.006
Cochran-Armitage test	P=0.007		
Fisher exact test		P=0.123	P=0.007
Liver: Hepatocellular Adenoma or Neoplastic Nodule			
Overall rates	2/49 (4%)	2/50 (4%)	4/48 (8%)
Adjusted rates	11.2%	14.3%	14.9%
Terminal rates	0/9 (0%)	2/14 (14%)	1/16 (6%)
First incidence (days)	698	799 (T)	615
Life table tests	P=0.359	P=0.586N	P=0.434
Logistic regression tests	P=0.248	P=0.661N	P=0.333
Cochran-Armitage test	P=0.237		
Fisher exact test		P=0.684N	P=0.329
Pancreatic Islets: Adenoma			
Overall rates	1/47 (2%)	0/41 (0%)	2/43 (5%)
Adjusted rates	12.5%	0.0%	9.9%
Terminal rates	1/8 (13%)	0/13 (0%)	1/13 (8%)
First incidence (days)	799 (T)	- ^e	617
Life table tests	P=0.387	P=0.403N	P=0.612
Logistic regression tests	P=0.308	P=0.403N	P=0.479
Cochran-Armitage test	P=0.304		
Fisher exact test		P=0.534N	P=0.466

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Lesions in Male Rats

A-25

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the Lifetime Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Pancreatic Islets: Adenoma or Carcinoma			
Overall rates	2/47 (4%)	0/41 (0%)	2/43 (5%)
Adjusted rates	25.0%	0.0%	9.9%
Terminal rates	2/8 (25%)	0/13 (0%)	1/13 (8%)
First incidence (days)	799 (T)	—	617
Life table tests	P=0.650	P=0.135N	P=0.560N
Logistic regression tests	P=0.544	P=0.135N	P=0.683
Cochran-Armitage test	P=0.531		
Fisher exact test		P=0.282N	P=0.657
Pituitary Gland (Pars Distalis): Adenoma			
Overall rates	12/47 (26%)	11/50 (22%)	10/49 (20%)
Adjusted rates	53.6%	42.8%	42.1%
Terminal rates	3/9 (33%)	3/14 (21%)	4/16 (25%)
First incidence (days)	568	558	697
Life table tests	P=0.174N	P=0.334N	P=0.160N
Logistic regression tests	P=0.307N	P=0.419N	P=0.324N
Cochran-Armitage test	P=0.344N		
Fisher exact test		P=0.432N	P=0.362N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma			
Overall rates	12/47 (26%)	12/50 (24%)	10/49 (20%)
Adjusted rates	53.6%	45.8%	42.1%
Terminal rates	3/9 (33%)	3/14 (21%)	4/16 (25%)
First incidence (days)	568	558	697
Life table tests	P=0.159N	P=0.411N	P=0.160N
Logistic regression tests	P=0.287N	P=0.509N	P=0.324N
Cochran-Armitage test	P=0.325N		
Fisher exact test		P=0.524N	P=0.362N
Preputial Gland: Carcinoma			
Overall rates	1/48 (2%)	6/49 (12%)	1/48 (2%)
Adjusted rates	2.3%	22.5%	2.5%
Terminal rates	0/9 (0%)	1/14 (7%)	0/16 (0%)
First incidence (days)	586	527	628
Life table tests	P=0.361N	P=0.090	P=0.753N
Logistic regression tests	P=0.440N	P=0.058	P=0.750
Cochran-Armitage test	P=0.425N		
Fisher exact test		P=0.059	P=0.753N
Preputial Gland: Adenoma or Carcinoma			
Overall rates	2/48 (4%)	7/49 (14%)	2/48 (4%)
Adjusted rates	4.4%	28.5%	8.6%
Terminal rates	0/9 (0%)	2/14 (14%)	1/16 (6%)
First incidence (days)	429	527	628
Life table tests	P=0.331N	P=0.134	P=0.632N
Logistic regression tests	P=0.454N	P=0.078	P=0.673
Cochran-Armitage test	P=0.436N		
Fisher exact test		P=0.084	P=0.692N

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A-26

Talc, NTP TR 421

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the Lifetime Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Skin: Keratoacanthoma or Squamous Cell Carcinoma			
Overall rates	0/49 (0%)	3/50 (6%)	2/50 (4%)
Adjusted rates	0.0%	13.5%	6.6%
Terminal rates	0/9 (0%)	1/14 (7%)	0/16 (0%)
First incidence (days)	-	663	594
Life table tests	P=0.414	P=0.161	P=0.331
Logistic regression tests	P=0.323	P=0.128	P=0.239
Cochran-Armitage test	P=0.319		
Fisher exact test		P=0.125	P=0.253
Testes: Adenoma			
Overall rates	31/49 (63%)	39/50 (78%)	36/50 (72%)
Adjusted rates	100.0%	100.0%	97.0%
Terminal rates	9/9 (100%)	14/14 (100%)	15/16 (94%)
First incidence (days)	551	544	609
Life table tests	P=0.198N	P=0.524	P=0.245N
Logistic regression tests	P=0.333	P=0.056	P=0.268
Cochran-Armitage test	P=0.295		
Fisher exact test		P=0.082	P=0.238
Thyroid Gland (C-cell): Adenoma			
Overall rates	3/45 (7%)	4/46 (9%)	3/46 (7%)
Adjusted rates	24.5%	28.6%	14.5%
Terminal rates	2/9 (22%)	4/14 (29%)	2/16 (13%)
First incidence (days)	682	799 (I)	614
Life table tests	P=0.348N	P=0.620N	P=0.476N
Logistic regression tests	P=0.511N	P=0.641	P=0.625N
Cochran-Armitage test	P=0.560N		
Fisher exact test		P=0.512	P=0.651N
Thyroid Gland (C-cell): Adenoma or Carcinoma			
Overall rates	3/45 (7%)	5/46 (11%)	3/46 (7%)
Adjusted rates	24.5%	33.0%	14.5%
Terminal rates	2/9 (22%)	4/14 (29%)	2/16 (13%)
First incidence (days)	682	787	614
Life table tests	P=0.296N	P=0.568	P=0.476N
Logistic regression tests	P=0.467N	P=0.502	P=0.625N
Cochran-Armitage test	P=0.523N		
Fisher exact test		P=0.369	P=0.651N
All Organs: Mononuclear Cell Leukemia			
Overall rates	24/49 (49%)	21/50 (42%)	23/50 (46%)
Adjusted rates	70.3%	59.9%	62.5%
Terminal rates	3/9 (33%)	4/14 (29%)	6/16 (38%)
First incidence (days)	334	529	492
Life table tests	P=0.298N	P=0.232N	P=0.269N
Logistic regression tests	P=0.501N	P=0.317N	P=0.479N
Cochran-Armitage test	P=0.486N		
Fisher exact test		P=0.310N	P=0.462N

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Lesions in Male Rats

A-27

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the Lifetime Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
All Organs: Benign Tumors			
Overall rates	42/49 (86%)	45/50 (90%)	45/50 (90%)
Adjusted rates	100.0%	100.0%	100.0%
Terminal rates	9/9 (100%)	14/14 (100%)	16/16 (100%)
First incidence (days)	429	544	594
Life table tests	P=0.161N	P=0.314N	P=0.153N
Logistic regression tests	P=0.463	P=0.430	P=0.480
Cochran-Armitage test	P=0.353		
Fisher exact test		P=0.365	P=0.365
All Organs: Malignant Tumors			
Overall rates	34/49 (69%)	34/50 (68%)	34/50 (68%)
Adjusted rates	88.4%	80.9%	80.0%
Terminal rates	6/9 (67%)	7/14 (50%)	9/16 (56%)
First incidence (days)	334	527	248
Life table tests	P=0.222N	P=0.308N	P=0.216N
Logistic regression tests	P=0.534N	P=0.539N	P=0.571N
Cochran-Armitage test	P=0.505N		
Fisher exact test		P=0.527N	P=0.527N
All Organs: Benign or Malignant Tumors			
Overall rates	48/49 (98%)	49/50 (98%)	50/50 (100%)
Adjusted rates	100.0%	100.0%	100.0%
Terminal rates	9/9 (100%)	14/14 (100%)	16/16 (100%)
First incidence (days)	334	527	248
Life table tests	P=0.154N	P=0.241N	P=0.139N
Logistic regression tests	P=0.337	P=0.771	P=0.506
Cochran-Armitage test	P=0.348		
Fisher exact test		P=0.747	P=0.495

(T) Terminal sacrifice

^a Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.

^b Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality

^c Observed incidence at terminal kill

^d Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.

^e Not applicable; no tumors in animal group

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A-28

Talc, NTP TR 421

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Lifetime Inhalation Study of Talc^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Moribund	23	19	20
Natural deaths	18	17	14
Survivors			
Died last week of study	1	2	3
Terminal sacrifice	8	12	13
Animals examined microscopically	49	50	50
Alimentary System			
Esophagus	(49)	(50)	(49)
Inflammation			1 (2%)
Intestine large, cecum	(42)	(38)	(37)
Hemorrhage		1 (3%)	
Inflammation	9 (21%)	2 (5%)	5 (14%)
Parasite metazoan	3 (7%)	4 (11%)	4 (11%)
Ulcer	1 (2%)		
Intestine large, colon	(43)	(43)	(46)
Hyperplasia, lymphoid	1 (2%)		
Inflammation	1 (2%)		1 (2%)
Mineralization			1 (2%)
Parasite metazoan	2 (5%)	1 (2%)	1 (2%)
Intestine large, rectum	(38)	(41)	(34)
Inflammation	6 (16%)	1 (2%)	1 (3%)
Metaplasia, squamous, focal			1 (3%)
Parasite metazoan		2 (5%)	
Intestine small, duodenum	(48)	(44)	(46)
Inflammation			1 (2%)
Mineralization	1 (2%)		
Necrosis, focal	1 (2%)		
Ulcer	1 (2%)	1 (2%)	
Intestine small, ileum	(39)	(34)	(35)
Hyperplasia, lymphoid		1 (3%)	2 (6%)
Lymphatic, ectasia		1 (3%)	
Liver	(49)	(50)	(48)
Angiectasis, focal	1 (2%)		
Atrophy	1 (2%)		
Basophilic focus	18 (37%)	18 (36%)	19 (40%)
Clear cell focus	3 (6%)	7 (14%)	4 (8%)
Congestion		1 (2%)	
Degeneration, cystic	9 (18%)	17 (34%)	9 (19%)
Degeneration, diffuse			1 (2%)
Eosinophilic focus	2 (4%)	7 (14%)	7 (15%)
Fatty change	16 (33%)	14 (28%)	12 (25%)
Fibrosis	1 (2%)		
Hematocyst		1 (2%)	
Hyperplasia, focal			1 (2%)
Inflammation, granulomatous, focal	3 (6%)	1 (2%)	
Inflammation, necrotizing, focal			1 (2%)
Necrosis, focal	3 (6%)		1 (2%)
Thrombosis	1 (2%)		
Bile duct, hyperplasia	39 (80%)	46 (92%)	44 (92%)

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Lesions in Male Rats

A-29

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Lifetime Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Alimentary System (continued)			
Liver (continued)			
Centrilobular, atrophy	9 (18%)	4 (8%)	7 (15%)
Centrilobular, degeneration	8 (16%)	12 (24%)	9 (19%)
Centrilobular, degeneration, fatty			1 (2%)
Centrilobular, necrosis	5 (10%)		2 (4%)
Mesentery	(2)		(1)
Inflammation			1 (100%)
Pancreas	(48)	(46)	(47)
Lobules, atrophy	11 (23%)	7 (15%)	8 (17%)
Salivary glands	(49)	(50)	(50)
Inflammation	1 (2%)		
Necrosis			1 (2%)
Stomach, forestomach	(49)	(47)	(47)
Hyperkeratosis			1 (2%)
Inflammation	1 (2%)		
Mineralization	1 (2%)	4 (9%)	1 (2%)
Ulcer	5 (10%)	5 (11%)	8 (17%)
Stomach, glandular	(49)	(47)	(47)
Mineralization	6 (12%)	6 (13%)	6 (13%)
Ulcer	3 (6%)	3 (6%)	2 (4%)
Cardiovascular System			
Blood vessel	(4)	(5)	(5)
Aorta, mineralization	3 (75%)	5 (100%)	4 (80%)
Mesenteric artery, aneurysm			2 (40%)
Mesenteric artery, inflammation			1 (20%)
Mesenteric artery, mineralization	3 (75%)	5 (100%)	3 (60%)
Mesenteric artery, thrombosis	1 (25%)	1 (20%)	1 (20%)
Heart	(49)	(50)	(50)
Cardiomyopathy	42 (86%)	47 (94%)	50 (100%)
Atrium, thrombosis	9 (18%)	5 (10%)	11 (22%)
Epicardium, hyperplasia	1 (2%)		
Myocardium, inflammation		1 (2%)	
Myocardium, mineralization	2 (4%)	6 (12%)	5 (10%)
Endocrine System			
Adrenal gland, cortex	(49)	(49)	(48)
Degeneration	1 (2%)		
Degeneration, fatty	8 (16%)		2 (4%)
Degeneration, focal	1 (2%)		
Hyperplasia, diffuse			2 (4%)
Hyperplasia, focal	11 (22%)	4 (8%)	9 (19%)
Necrosis, focal	1 (2%)		
Adrenal gland, medulla	(49)	(48)	(47)
Hyperplasia	19 (39%)	8 (17%)	8 (17%)
Bilateral, hyperplasia	1 (2%)		1 (2%)
Islets, pancreatic	(47)	(41)	(43)
Hyperplasia			1 (2%)
Parathyroid gland	(45)	(45)	(46)
Hyperplasia	6 (13%)	11 (24%)	12 (26%)
Bilateral, hyperplasia	1 (2%)		

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A-30

Talc, NTP TR 421

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Lifetime Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Endocrine System (continued)			
Pituitary gland	(47)	(50)	(49)
Angiectasis, focal		1 (2%)	
Cyst		1 (2%)	1 (2%)
Pars distalis, hyperplasia	8 (17%)	8 (16%)	7 (14%)
Pars nervosa, hyperplasia		1 (2%)	
Thyroid gland	(45)	(46)	(46)
C-cell, hyperplasia	5 (11%)	7 (15%)	2 (4%)
General Body System			
None			
Genital System			
Epididymis	(49)	(50)	(49)
Spermatocele		1 (2%)	
Preputial gland	(48)	(49)	(48)
Hyperplasia	3 (6%)		1 (2%)
Inflammation	7 (15%)	2 (4%)	5 (10%)
Prostate	(49)	(45)	(48)
Atrophy	1 (2%)		1 (2%)
Inflammation	22 (45%)	14 (31%)	19 (40%)
Seminal vesicle	(49)	(48)	(47)
Atrophy	1 (2%)		
Inflammation	1 (2%)		
Testes	(49)	(50)	(50)
Atrophy	14 (29%)	11 (22%)	16 (32%)
Hyperplasia, lymphoid		2 (4%)	
Hyperplasia, lymphoid, focal			1 (2%)
Interstitial cell, hyperplasia	2 (4%)	1 (2%)	3 (6%)
Serosa, proliferation			1 (2%)
Hematopoietic System			
Bone marrow	(48)	(48)	(47)
Atrophy			2 (4%)
Atrophy, focal		1 (2%)	
Inflammation		1 (2%)	
Myelofibrosis		1 (2%)	1 (2%)
Myeloid cell, hyperplasia	2 (4%)	3 (6%)	6 (13%)
Lymph node	(49)	(50)	(50)
Hemorrhage, chronic		1 (2%)	
Pancreatic, atrophy	1 (2%)		
Pancreatic, hyperplasia, lymphoid	1 (2%)		
Lymph node, bronchial	(41)	(48)	(49)
Atrophy	2 (5%)		
Hemorrhage		1 (2%)	
Hemorrhage, acute	1 (2%)		
Hemorrhage, chronic	4 (10%)		
Hyperplasia, histiocytic		44 (92%)	46 (94%)
Lymph node, mandibular	(46)	(48)	(47)
Hemorrhage		1 (2%)	
Hyperplasia, lymphoid		2 (4%)	
Hyperplasia, plasma cell		2 (4%)	5 (11%)
Inflammation, chronic active			2 (4%)

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Lesions in Male Rats

A-31

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Lifetime Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Hematopoietic System (continued)			
Lymph node, mediastinal	(48)	(49)	(47)
Atrophy	1 (2%)		
Hemorrhage		3 (6%)	
Hemorrhage, acute	1 (2%)		
Hemorrhage, chronic	6 (13%)		
Hyperplasia, histiocytic		40 (82%)	43 (91%)
Pigmentation, hemosiderin	1 (2%)		
Lymph node, mesenteric	(49)	(48)	(47)
Atrophy	1 (2%)		
Hemorrhage		2 (4%)	
Hemorrhage, acute	1 (2%)		
Hyperplasia, lymphoid	1 (2%)	2 (4%)	3 (6%)
Hyperplasia, plasma cell			1 (2%)
Inflammation, chronic active			1 (2%)
Spleen	(49)	(50)	(48)
Atrophy	1 (2%)		2 (4%)
Autolysis			1 (2%)
Congestion, chronic	1 (2%)		
Cyst			1 (2%)
Fibrosis		1 (2%)	
Fibrosis, focal		5 (10%)	2 (4%)
Hematopoietic cell proliferation	1 (2%)	2 (4%)	3 (6%)
Hyperplasia, histiocytic		1 (2%)	
Hyperplasia, lymphoid		1 (2%)	1 (2%)
Infarct	3 (6%)		
Inflammation, granulomatous, focal	1 (2%)		1 (2%)
Thymus	(48)	(40)	(43)
Atrophy		2 (5%)	
Cyst	1 (2%)		
Integumentary System			
Mammary gland	(45)	(48)	(50)
Galactocoele	1 (2%)		
Skin	(48)	(50)	(50)
Cyst epithelial inclusion		1 (2%)	
Subcutaneous tissue, inflammation			1 (2%)
Tail, necrosis	1 (2%)		
Musculoskeletal System			
Bone	(49)	(50)	(50)
Fibrous osteodystrophy	3 (6%)	4 (8%)	5 (10%)
Coccygeal, necrosis	1 (2%)		
Pelvis, fracture		1 (2%)	
Nervous System			
Brain	(49)	(50)	(50)
Compression	5 (10%)	2 (4%)	2 (4%)
Hemorrhage		1 (2%)	
Infarct			1 (2%)
Necrosis, focal	1 (2%)	2 (4%)	
Spinal cord			(1)
Degeneration			1 (100%)

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A-32

Talc, NTP TR 421

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Lifetime Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Respiratory System			
Larynx	(48)	(49)	(49)
Inflammation, suppurative	6 (13%)		
Lung	(49)	(50)	(50)
Congestion	1 (2%)		
Crystals, focal	1 (2%)		
Cyst			3 (6%)
Hemorrhage, chronic	2 (4%)		
Infarct	1 (2%)		
Inflammation, granulomatous	2 (4%)	50 (100%)	49 (98%)
Inflammation, suppurative		2 (4%)	
Mineralization		4 (8%)	
Alveolar epithelium, hyperplasia	5 (10%)	26 (52%)	38 (76%)
Alveolus, hemorrhage, focal	1 (2%)		
Alveolus, metaplasia, squamous			2 (4%)
Artery, thrombosis	1 (2%)		
Interstitial, fibrosis, focal	1 (2%)	16 (32%)	33 (66%)
Interstitial, mineralization	2 (4%)	1 (2%)	4 (8%)
Peribronchial, hyperplasia, histiocytic		12 (24%)	8 (16%)
Nose	(49)	(48)	(47)
Inflammation, suppurative	2 (4%)	1 (2%)	
Lumen, foreign body	1 (2%)		
Lumen, hemorrhage			1 (2%)
Mucosa, inflammation, suppurative	4 (8%)	5 (10%)	2 (4%)
Nasolacrimal duct, inflammation, suppurative		1 (2%)	
Respiratory epithelium, hyperplasia		3 (6%)	14 (30%)
Trachea	(49)	(50)	(48)
Inflammation, suppurative	3 (6%)		1 (2%)
Special Senses System			
Eye	(3)	(2)	(2)
Cataract	1 (33%)	1 (50%)	2 (100%)
Inflammation, chronic			1 (50%)
Cornea, inflammation, necrotizing			1 (50%)
Cornea, necrosis	1 (33%)		
Lens, cataract	1 (33%)		
Retina, degeneration	2 (67%)	1 (50%)	1 (50%)
Urinary System			
Kidney	(49)	(49)	(48)
Calculus micro observation only			1 (2%)
Cyst	3 (6%)		1 (2%)
Hydronephrosis		1 (2%)	1 (2%)
Nephropathy	45 (92%)	47 (96%)	43 (90%)
Medulla, inflammation		1 (2%)	1 (2%)
Renal tubule, necrosis		1 (2%)	
Ureter			(1)
Calculus micro observation only			1 (100%)
Urethra			(1)
Fibrosis			1 (100%)
Urinary bladder	(49)	(48)	(47)
Calculus gross observation			1 (2%)
Inflammation	1 (2%)		
Mucosa, hyperplasia			1 (2%)

^a Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

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B-1

APPENDIX B SUMMARY OF LESIONS IN FEMALE RATS IN THE LIFETIME INHALATION STUDY OF TALC

TABLE B1	Summary of the Incidence of Neoplasms in Female Rats in the Lifetime Inhalation Study of Talc	B-2
TABLE B2	Individual Animal Tumor Pathology of Female Rats in the Lifetime Inhalation Study of Talc	B-6
TABLE B3	Statistical Analysis of Primary Neoplasms in Female Rats in the Lifetime Inhalation Study of Talc	B-24
TABLE B4	Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Lifetime Inhalation Study of Talc	B-29

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B-2

Talc, NTP TR 421

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the Lifetime Inhalation Study of Talc^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Moribund	28	17	27
Natural deaths	11	19	14
Survivors			
Terminal sacrifice	11	13	9
Missing		1	
Animals examined microscopically	50	49	50
Alimentary System			
Intestine small, ileum	(44)	(32)	(38)
Liver	(50)	(48)	(50)
Granulosa-theca tumor malignant, metastatic, ovary			1 (2%)
Hepatocellular carcinoma		1 (2%)	
Neoplastic nodule		3 (6%)	1 (2%)
Pancreas	(50)	(46)	(49)
Pharynx			(1)
Squamous cell carcinoma			1 (100%)
Salivary glands	(50)	(48)	(50)
Fibrosarcoma			1 (2%)
Sarcoma		1 (2%)	
Stomach, forestomach	(50)	(45)	(49)
Stomach, glandular	(50)	(47)	(50)
Tongue		(2)	
Sarcoma, metastatic		1 (50%)	
Squamous cell papilloma		1 (50%)	
Tooth		(1)	
Adamantinoma benign		1 (100%)	
Cardiovascular System			
Heart	(50)	(48)	(50)
Granulosa-theca tumor malignant, metastatic, ovary			1 (2%)
Endocrine System			
Adrenal gland, cortex	(50)	(47)	(49)
Granulosa-theca tumor malignant, metastatic, ovary			1 (2%)
Adrenal gland, medulla	(48)	(47)	(49)
Granulosa-theca tumor malignant, metastatic, ovary			1 (2%)
Pheochromocytoma malignant		1 (2%)	7 (14%)
Pheochromocytoma benign	13 (27%)	10 (21%)	11 (22%)
Bilateral, pheochromocytoma malignant			3 (6%)
Bilateral, pheochromocytoma benign		4 (9%)	7 (14%)
Islets, pancreatic	(50)	(45)	(49)
Adenoma	1 (2%)	1 (2%)	1 (2%)
Parathyroid gland	(43)	(42)	(47)
Pituitary gland	(50)	(47)	(50)
Pars distalis, adenoma	19 (38%)	18 (38%)	21 (42%)
Pars distalis, carcinoma	3 (6%)	3 (6%)	2 (4%)

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Board Draft

Lesions in Female Rats

B-3

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the Lifetime Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Endocrine System (continued)			
Thyroid gland	(50)	(47)	(49)
Bilateral, C-cell, carcinoma	1 (2%)		
C-cell, adenoma	5 (10%)		6 (12%)
C-cell, carcinoma	2 (4%)	2 (4%)	2 (4%)
Follicular cell, adenoma		1 (2%)	
General Body System			
None			
Genital System			
Clitoral gland	(47)	(44)	(46)
Adenoma			1 (2%)
Carcinoma	2 (4%)		1 (2%)
Ovary	(50)	(47)	(50)
Granulosa cell tumor malignant	1 (2%)		
Granulosa cell tumor benign		2 (4%)	
Granulosa-theca tumor benign		1 (2%)	
Bilateral, granulosa-theca tumor malignant			1 (2%)
Uterus	(50)	(48)	(50)
Polyp stromal	5 (10%)	7 (15%)	4 (8%)
Sarcoma stromal		1 (2%)	
Hematopoietic System			
Bone marrow	(50)	(43)	(49)
Lymph node	(50)	(48)	(50)
Lymph node, bronchial	(46)	(47)	(47)
Adenocarcinoma, metastatic, thyroid gland	1 (2%)		
Squamous cell carcinoma, metastatic, lung			1 (2%)
Lymph node, mandibular	(47)	(46)	(47)
Sarcoma, metastatic		1 (2%)	
Lymph node, mediastinal	(47)	(44)	(47)
Adenocarcinoma, metastatic, thyroid gland	1 (2%)		
Carcinoma, metastatic, uncertain primary site			1 (2%)
Fibrosarcoma, metastatic, skin			1 (2%)
Granulosa-theca tumor malignant, metastatic, ovary			1 (2%)
Lymph node, mesenteric	(49)	(47)	(47)
Spleen	(50)	(48)	(50)
Thymus	(47)	(44)	(47)
Mixed tumor malignant		1 (2%)	
Myxoma		1 (2%)	
Schwannoma benign			1 (2%)
Thymoma benign	1 (2%)		
Integumentary System			
Mammary gland	(50)	(48)	(50)
Adenocarcinoma	2 (4%)		2 (4%)
Adenoma	1 (2%)	2 (4%)	2 (4%)
Fibroadenoma	11 (22%)	10 (21%)	13 (26%)
Fibroma	1 (2%)	1 (2%)	
Fibrosarcoma			1 (2%)

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B-4

Talc, NTP TR 421

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the Lifetime Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Integumentary System (continued)			
Skin	(50)	(49)	(50)
Keratoacanthoma		1 (2%)	1 (2%)
Subcutaneous tissue, fibrosarcoma		1 (2%)	1 (2%)
Musculoskeletal System			
Bone	(50)	(48)	(50)
Mandible, sarcoma	1 (2%)		
Mandible, sarcoma, metastatic		1 (2%)	
Skeletal muscle	(1)	(1)	
Liposarcoma		1 (100%)	
Nervous System			
Brain	(50)	(48)	(50)
Astrocytoma benign	1 (2%)		
Carcinoma, metastatic, pituitary gland	2 (4%)	1 (2%)	1 (2%)
Ependymoma malignant	1 (2%)		
Respiratory System			
Larynx	(50)	(48)	(48)
Adenocarcinoma, metastatic, thyroid gland	1 (2%)		
Lung	(50)	(48)	(50)
Adenocarcinoma, metastatic, multiple, mammary gland	1 (2%)		
Alveolar/bronchiolar adenoma	1 (2%)		8 (16%)
Alveolar/bronchiolar adenoma, multiple			1 (2%)
Alveolar/bronchiolar carcinoma			4 (8%)
Alveolar/bronchiolar carcinoma, multiple			1 (2%)
Granulosa-theca tumor malignant, metastatic, ovary			1 (2%)
Squamous cell carcinoma			1 (2%)
Special Senses System			
None			
Urinary System			
Kidney	(49)	(47)	(49)
Lipoma		1 (2%)	
Urinary bladder	(50)	(45)	(50)
Systemic Lesions			
Multiple organs ^b	(50)	(49)	(50)
Leukemia mononuclear	13 (26%)	20 (41%)	18 (36%)
Lymphoma malignant lymphocytic		2 (4%)	
Lymphoma malignant mixed		1 (2%)	

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B-5

Lesions in Female Rats

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the Lifetime Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Tumor Summary			
Total animals with primary neoplasms ^c	44	47	49
Total primary neoplasms	85	100	124
Total animals with benign neoplasms	38	35	39
Total benign neoplasms	59	65	78
Total animals with malignant neoplasms	23	31	35
Total malignant neoplasms	26	35	46
Total animals with metastatic neoplasms	4	3	4
Total metastatic neoplasms	6	8	10
Total animals with malignant neoplasms, uncertain primary site			1

^a Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

^b Number of animals with any tissue examined microscopically

^c Primary tumors: all tumors except metastatic tumors

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Talc, NTP TR 421

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the Lifetime Inhalation Study of Talc: 0 mg/m³ (continued)

Number of Days on Study	3 3 3 5 5 5 5 5 6 6 6 6 6 6 7 7 7 7 7 7 7 7
	7 9 9 5 6 8 8 9 2 3 4 7 7 8 9 1 1 2 3 6 6 6 6 7 7
	0 0 8 8 8 4 6 9 6 4 7 7 8 8 6 6 9 7 1 2 6 7 8 2 9
Carcass ID Number	3 4 3 3 3 3 4 4 3 3 3 3 4 3 3 3 3 4 3 3 3 3 3 4
	3 3 7 0 5 5 3 2 5 8 0 7 0 0 5 2 0 0 9 8 5 2 2 3 0
	5 0 7 6 0 7 2 9 8 4 2 6 2 8 9 7 5 6 7 2 1 8 6 1 8
	1 1
Genital System	
Clitoral gland	M + + + + + + + + + + + + + + + + + M + + +
Carcinoma	
Ovary	+ +
Granulosa cell tumor malignant	
Uterus	+ +
Polyp stromal	X
Hematopoietic System	
Bone marrow	+ +
Lymph node	+ +
Lymph node, bronchial	+ +
Adenocarcinoma, metastatic, thyroid gland	
	X
Lymph node, mandibular	+ + M + + + + + + + + + + + + + + + + + M + + + + +
Lymph node, mediastinal	M +
Adenocarcinoma, metastatic, thyroid gland	
	X
Lymph node, mesenteric	M +
Spleen	+ +
Thymus	M M M +
Thymoma benign	
Integumentary System	
Mammary gland	+ +
Adenocarcinoma	X
Adenoma	X
Fibroadenoma	
Fibroma	X X
Skin	+ +
Musculoskeletal System	
Bone	+ +
Mandibular, sarcoma	
Skeletal muscle	X
Nervous System	
Brain	+ +
Astrocytoma benign	
Carcinoma, metastatic, pituitary gland	X
Ependymoma malignant	X

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[illegible]

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[illegible]

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TABLE B2
Individual Animal Tumor Pathology of Female Rats in the Lifetime Inhalation Study of Talc: 18 mg/m³ (continued)

Number of Days on Study	5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7
	3 5 8 9 1 3 4 6 6 7 7 7 8 9 9 0 1 1 1 2 3 4 4 4 7
	6 8 6 4 5 3 6 0 1 5 6 8 4 3 7 6 0 6 7 4 9 0 6 7 3
Carcass ID Number	1 2 2 2 1 2 2 2 1 2 2 2 2 2 1 2 1 2 2 2 1 2 1 2 2
	6 8 1 1 8 8 3 8 8 8 0 5 0 4 8 0 6 8 3 5 8 6 9 3 0
	7 0 6 2 3 5 0 1 6 8 7 5 8 0 9 6 0 2 6 6 1 3 2 9 9
	1 1
Integumentary System	
Mammary gland	+ +
Adenocarcinoma	
Adenoma	
Fibroadenoma	X X X X X
Fibrosarcoma	
Skin	+ +
Keratoacanthoma	X
Subcutaneous tissue, fibrosarcoma	X
Musculoskeletal System	
Bone	+ +
Nervous System	
Brain	+ +
Carcinoma, metastatic, pituitary gland	X
Respiratory System	
Larynx	+ + + + + I + I + + + + + + + + + + + + + + +
Lung	+ +
Alveolar/bronchiolar adenoma	
Alveolar/bronchiolar adenoma, multiple	X X
Alveolar/bronchiolar carcinoma	
Alveolar/bronchiolar carcinoma, multiple	
Granulosa-theca tumor malignant, metastatic, ovary	X
Squamous cell carcinoma	X
Nose	+ + + + + + + + + + + + + + + A + + + + + + +
Trachea	+ +
Special Senses System	
Eye	
Harderian gland	+ + + + + + + + + + +
Lacrimal gland	
Urinary System	
Kidney	+ + A +
Urinary bladder	+ +
Systemic Lesions	
Multiple organs	+ +
Leukemia mononuclear	X X X X X X X X X X X X X X

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Talc, NTP TR 421

TABLE B3

Statistical Analysis of Primary Neoplasms in Female Rats in the Lifetime Inhalation Study of Talc

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Adrenal Medulla: Benign Pheochromocytoma			
Overall rates ^a	13/48 (27%)	14/47 (30%)	18/49 (37%)
Adjusted rates ^b	61.3%	59.7%	82.5%
Terminal rates ^c	5/11 (45%)	5/13 (38%)	6/9 (67%)
First incidence (days)	678	705	697
Life table tests ^d	P=0.135	P=0.529	P=0.183
Logistic regression tests ^d	P=0.185	P=0.541	P=0.225
Cochran-Armitage test ^d	P=0.180		
Fisher exact test ^d		P=0.474	P=0.212
Adrenal Medulla: Malignant Pheochromocytoma			
Overall rates	0/48 (0%)	1/47 (2%)	10/49 (20%)
Adjusted rates	0.0%	7.1%	56.9%
Terminal rates	0/11 (0%)	0/13 (0%)	3/9 (33%)
First incidence (days)	- ^e	849	784
Life table tests	P<0.001	P=0.531	P=0.002
Logistic regression tests	P<0.001	P=0.509	P=0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P=0.495	P<0.001
Adrenal Medulla: Benign or Malignant Pheochromocytoma			
Overall rates	13/48 (27%)	14/47 (30%)	23/49 (47%)
Adjusted rates	61.3%	59.7%	95.2%
Terminal rates	5/11 (45%)	5/13 (38%)	8/9 (89%)
First incidence (days)	678	705	697
Life table tests	P=0.016	P=0.529	P=0.033
Logistic regression tests	P=0.014	P=0.541	P=0.024
Cochran-Armitage test	P=0.021		
Fisher exact test		P=0.474	P=0.034
Liver: Neoplastic Nodule			
Overall rates	0/50 (0%)	3/48 (6%)	1/50 (2%)
Adjusted rates	0.0%	13.6%	10.0%
Terminal rates	0/11 (0%)	0/13 (0%)	0/9 (0%)
First incidence (days)	-	724	857
Life table tests	P=0.550	P=0.114	P=0.464
Logistic regression tests	P=0.561	P=0.117	P=0.496
Cochran-Armitage test	P=0.556		
Fisher exact test		P=0.114	P=0.500
Liver: Neoplastic Nodule or Hepatocellular Carcinoma			
Overall rates	0/50 (0%)	4/48 (8%)	1/50 (2%)
Adjusted rates	0.0%	20.2%	10.0%
Terminal rates	0/11 (0%)	1/13 (8%)	0/9 (0%)
First incidence (days)	-	724	857
Life table tests	P=0.575	P=0.066	P=0.464
Logistic regression tests	P=0.602	P=0.060	P=0.496
Cochran-Armitage test	P=0.599		
Fisher exact test		P=0.054	P=0.500

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Lesions in Female Rats

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TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the Lifetime Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Lung: Alveolar/bronchiolar Adenoma			
Overall rates	1/50 (2%)	0/48 (0%)	9/50 (18%)
Adjusted rates	4.5%	0.0%	40.8%
Terminal rates	0/11 (0%)	0/13 (0%)	1/9 (11%)
First incidence (days)	805	716	716
Life table tests	P<0.001	P=0.529N	P=0.015
Logistic regression tests	P<0.001	P=0.503N	P=0.010
Cochran-Armitage test	P<0.001		
Fisher exact test		P=0.510N	P=0.008
Lung: Alveolar/bronchiolar Carcinoma			
Overall rates	0/50 (0%)	0/48 (0%)	5/50 (10%)
Adjusted rates	0.0%	0.0%	41.7%
Terminal rates	0/11 (0%)	0/13 (0%)	3/9 (33%)
First incidence (days)	—	—	828
Life table tests	P=0.002	—	P=0.027
Logistic regression tests	P=0.003	—	P=0.028
Cochran-Armitage test	P=0.004	—	
Fisher exact test		—	P=0.028
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rates	1/50 (2%)	0/48 (0%)	13/50 (26%)
Adjusted rates	4.5%	0.0%	65.8%
Terminal rates	0/11 (0%)	0/13 (0%)	4/9 (44%)
First incidence (days)	805	—	716
Life table tests	P<0.001	P=0.529N	P=0.001
Logistic regression tests	P<0.001	P=0.503N	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P=0.510N	P<0.001
Mammary Gland: Fibroadenoma			
Overall rates	11/50 (22%)	10/49 (20%)	13/50 (26%)
Adjusted rates	47.6%	41.4%	64.0%
Terminal rates	2/11 (18%)	3/13 (23%)	4/9 (44%)
First incidence (days)	634	482	678
Life table tests	P=0.304	P=0.489N	P=0.394
Logistic regression tests	P=0.363	P=0.508N	P=0.428
Cochran-Armitage test	P=0.343		
Fisher exact test		P=0.521N	P=0.408
Mammary Gland: Fibroma, Fibroadenoma, or Adenoma			
Overall rates	13/50 (26%)	13/49 (27%)	15/50 (30%)
Adjusted rates	54.7%	59.0%	68.6%
Terminal rates	3/11 (27%)	6/13 (46%)	4/9 (44%)
First incidence (days)	634	482	678
Life table tests	P=0.314	P=0.544N	P=0.404
Logistic regression tests	P=0.394	P=0.585	P=0.434
Cochran-Armitage test	P=0.371		
Fisher exact test		P=0.567	P=0.412

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B-26

Talc, NTP TR 421

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the Lifetime Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Mammary Gland: Fibroma, Fibroadenoma, Adenoma, or Adenocarcinoma			
Overall rates	15/50 (30%)	13/49 (27%)	16/50 (32%)
Adjusted rates	56.6%	59.0%	70.1%
Terminal rates	3/11 (27%)	6/13 (46%)	4/9 (44%)
First incidence (days)	370	482	678
Life table tests	P=0.378	P=0.386N	P=0.494
Logistic regression tests	P=0.457	P=0.425N	P=0.531
Cochran-Armitage test	P=0.430		
Fisher exact test		P=0.437N	P=0.500
Pituitary Gland (Pars Distalis): Adenoma			
Overall rates	19/50 (38%)	18/47 (38%)	21/50 (42%)
Adjusted rates	62.1%	60.5%	78.3%
Terminal rates	3/11 (27%)	3/13 (23%)	4/9 (44%)
First incidence (days)	568	697	633
Life table tests	P=0.360	P=0.512N	P=0.425
Logistic regression tests	P=0.409	P=0.557N	P=0.457
Cochran-Armitage test	P=0.380		
Fisher exact test		P=0.571	P=0.419
Pituitary Gland (Pars Distalis): Carcinoma			
Overall rates	3/50 (6%)	3/47 (6%)	2/50 (4%)
Adjusted rates	17.1%	12.2%	5.6%
Terminal rates	1/11 (9%)	1/13 (8%)	0/9 (0%)
First incidence (days)	696	566	676
Life table tests	P=0.438N	P=0.636N	P=0.506N
Logistic regression tests	P=0.427N	P=0.634	P=0.497N
Cochran-Armitage test	P=0.418N		
Fisher exact test		P=0.631	P=0.500N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma			
Overall rates	22/50 (44%)	21/47 (45%)	23/50 (46%)
Adjusted rates	69.8%	66.2%	79.5%
Terminal rates	4/11 (36%)	4/13 (31%)	4/9 (44%)
First incidence (days)	568	566	633
Life table tests	P=0.429	P=0.502N	P=0.488
Logistic regression tests	P=0.506	P=0.570N	P=0.545
Cochran-Armitage test	P=0.471		
Fisher exact test		P=0.554	P=0.500
Thyroid Gland (C-cell): Adenoma			
Overall rates	5/50 (10%)	0/47 (0%)	6/49 (12%)
Adjusted rates	33.5%	0.0%	34.0%
Terminal rates	2/11 (18%)	0/13 (0%)	2/9 (22%)
First incidence (days)	805	-	678
Life table tests	P=0.253	P=0.030N	P=0.467
Logistic regression tests	P=0.283	P=0.029N	P=0.505
Cochran-Armitage test	P=0.276		
Fisher exact test		P=0.033N	P=0.486

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Lesions in Female Rats

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TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the Lifetime Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Thyroid Gland (C-cell): Carcinoma			
Overall rates	3/50 (6%)	2/47 (4%)	2/49 (4%)
Adjusted rates	11.1%	12.2%	4.9%
Terminal rates	0/11 (0%)	0/13 (0%)	0/9 (0%)
First incidence (days)	677	818	675
Life table tests	P=0.430N	P=0.507N	P=0.493N
Logistic regression tests	P=0.462N	P=0.516N	P=0.533N
Cochran-Armitage test	P=0.463N		
Fisher exact test		P=0.530N	P=0.510N
Thyroid Gland (C-cell): Adenoma or Carcinoma			
Overall rates	8/50 (16%)	2/47 (4%)	8/49 (16%)
Adjusted rates	40.9%	12.2%	37.2%
Terminal rates	2/11 (18%)	0/13 (0%)	2/9 (22%)
First incidence (days)	677	818	675
Life table tests	P=0.418	P=0.051N	P=0.579
Logistic regression tests	P=0.435	P=0.048N	P=0.599N
Cochran-Armitage test	P=0.414		
Fisher exact test		P=0.056N	P=0.590
Uterus: Stromal Polyp			
Overall rates	5/50 (10%)	7/49 (14%)	4/50 (8%)
Adjusted rates	22.3%	34.4%	19.5%
Terminal rates	1/11 (9%)	3/13 (23%)	1/9 (11%)
First incidence (days)	398	678	678
Life table tests	P=0.439N	P=0.400	P=0.532N
Logistic regression tests	P=0.376N	P=0.372	P=0.505N
Cochran-Armitage test	P=0.386N		
Fisher exact test		P=0.365	P=0.500N
Uterus: Stromal Polyp or Stromal Sarcoma			
Overall rates	5/50 (10%)	8/49 (16%)	4/50 (8%)
Adjusted rates	22.3%	35.8%	19.5%
Terminal rates	1/11 (9%)	3/13 (23%)	1/9 (11%)
First incidence (days)	398	557	678
Life table tests	P=0.412N	P=0.298	P=0.532N
Logistic regression tests	P=0.360N	P=0.265	P=0.505N
Cochran-Armitage test	P=0.363N		
Fisher exact test		P=0.264	P=0.500N
All Organs: Mononuclear Cell Leukemia			
Overall rates	13/50 (26%)	20/49 (41%)	18/50 (36%)
Adjusted rates	45.7%	73.3%	60.1%
Terminal rates	1/11 (9%)	8/13 (62%)	3/9 (33%)
First incidence (days)	390	526	536
Life table tests	P=0.234	P=0.164	P=0.232
Logistic regression tests	P=0.226	P=0.084	P=0.152
Cochran-Armitage test	P=0.250		
Fisher exact test		P=0.088	P=0.194

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B-28

Talc, NTP TR 421

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the Lifetime Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
All Organs: Malignant Lymphoma			
Overall rates	0/50 (0%)	3/49 (6%)	0/50 (0%)
Adjusted rates	0.0%	10.3%	0.0%
Terminal rates	0/11 (0%)	0/13 (0%)	0/9 (0%)
First incidence (days)	-	724	-
Life table tests	P=0.525N	P=0.124	-
Logistic regression tests	P=0.497N	P=0.118	-
Cochran-Armitage test	P=0.499N		
Fisher exact test		P=0.117	-
All Organs: Benign Tumors			
Overall rates	38/50 (76%)	35/49 (71%)	39/50 (78%)
Adjusted rates	97.2%	96.9%	97.4%
Terminal rates	10/11 (91%)	12/13 (92%)	8/9 (89%)
First incidence (days)	398	482	558
Life table tests	P=0.338	P=0.350N	P=0.440
Logistic regression tests	P=0.544	P=0.312N	P=0.562N
Cochran-Armitage test	P=0.415		
Fisher exact test		P=0.387N	P=0.500
All Organs: Malignant Tumors			
Overall rates	23/50 (46%)	31/49 (63%)	35/50 (70%)
Adjusted rates	69.3%	85.8%	90.5%
Terminal rates	4/11 (36%)	9/13 (69%)	6/9 (67%)
First incidence (days)	370	526	536
Life table tests	P=0.054	P=0.189	P=0.061
Logistic regression tests	P=0.013	P=0.056	P=0.010
Cochran-Armitage test	P=0.016		
Fisher exact test		P=0.064	P=0.013
All Organs: Benign or Malignant Tumors			
Overall rates	44/50 (88%)	47/49 (96%)	49/50 (98%)
Adjusted rates	97.6%	97.9%	100.0%
Terminal rates	10/11 (91%)	12/13 (92%)	9/9 (100%)
First incidence (days)	370	482	536
Life table tests	P=0.248	P=0.447	P=0.279
Logistic regression tests	P=0.053	P=0.145	P=0.060
Cochran-Armitage test	P=0.049		
Fisher exact test		P=0.141	P=0.056

^a Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.

^b Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality

^c Observed incidence at terminal kill

^d Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.

^e Not applicable; no tumors in animal group

^f Value of statistic cannot be computed.

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Lesions in Female Rats

B-29

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Lifetime Inhalation Study of Talc^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Moribund	28	17	27
Natural deaths	11	19	14
Survivors			
Terminal sacrifice	11	13	9
Missing		1	
Animals examined microscopically	50	49	50
Alimentary System			
Intestine large, cecum	(46)	(34)	(43)
Hemorrhage, focal		1 (3%)	
Inflammation	11 (24%)	1 (3%)	6 (14%)
Parasite metazoan	7 (15%)	3 (9%)	6 (14%)
Ulcer	1 (2%)	1 (3%)	1 (2%)
Intestine large, colon	(48)	(41)	(45)
Inflammation		1 (2%)	2 (4%)
Parasite metazoan	2 (4%)	3 (7%)	3 (7%)
Intestine large, rectum	(38)	(37)	(41)
Inflammation	4 (11%)		
Parasite metazoan	2 (5%)	1 (3%)	1 (2%)
Intestine small, duodenum	(48)	(44)	(47)
Necrosis, focal	1 (2%)		
Intestine small, ileum	(44)	(32)	(38)
Hyperplasia, lymphoid	2 (5%)		
Liver	(50)	(48)	(50)
Atrophy		1 (2%)	1 (2%)
Basophilic focus	27 (54%)	17 (35%)	21 (42%)
Clear cell focus	1 (2%)	2 (4%)	1 (2%)
Cyst multilocular	1 (2%)		
Degeneration, cystic		2 (4%)	1 (2%)
Eosinophilic focus	2 (4%)	5 (10%)	4 (8%)
Fatty change	18 (36%)	18 (38%)	14 (28%)
Hematopoietic cell proliferation	1 (2%)		
Infiltration cellular, mononuclear cell			1 (2%)
Inflammation, granulomatous, focal	13 (26%)	3 (6%)	4 (8%)
Inflammation, necrotizing, focal		1 (2%)	
Inflammation, suppurative	1 (2%)		
Necrosis, focal	5 (10%)	1 (2%)	2 (4%)
Pigmentation, hemosiderin	1 (2%)		
Thrombosis			1 (2%)
Bile duct, hyperplasia	36 (72%)	38 (79%)	36 (72%)
Centrilobular, atrophy		2 (4%)	6 (12%)
Centrilobular, degeneration	10 (20%)	14 (29%)	10 (20%)
Centrilobular, necrosis	2 (4%)	2 (4%)	2 (4%)
Hepatocyte, atrophy, focal			1 (2%)
Serosa, thrombosis		3 (6%)	
Mesentery	(1)	(2)	
Granuloma	1 (100%)	1 (50%)	
Inflammation, chronic active		1 (50%)	

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Talc, NTP TR 421

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Lifetime Inhalation Study of Talc
 (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Alimentary System (continued)			
Pancreas	(50)	(46)	(49)
Hyperplasia, nodular	1 (2%)		
Inflammation		1 (2%)	
Lobules, atrophy	7 (14%)	7 (15%)	9 (18%)
Salivary glands	(50)	(48)	(50)
Inflammation	2 (4%)		
Stomach, forestomach	(50)	(45)	(49)
Hyperkeratosis	1 (2%)		1 (2%)
Inflammation	1 (2%)		2 (4%)
Mineralization		1 (2%)	
Ulcer	9 (18%)	4 (9%)	3 (6%)
Stomach, glandular	(50)	(47)	(50)
Erosion			1 (2%)
Inflammation	1 (2%)	1 (2%)	
Mineralization	2 (4%)	2 (4%)	2 (4%)
Ulcer	3 (6%)	2 (4%)	3 (6%)
Ulcer, multiple	1 (2%)		1 (2%)
Arteriole, muscularis, lamina propria, mineralization		1 (2%)	
Cardiovascular System			
Blood vessel	(3)	(3)	(1)
Aorta, mineralization		3 (100%)	1 (100%)
Mesenteric artery, aneurysm	1 (33%)		
Mesenteric artery, inflammation	3 (100%)		
Mesenteric artery, mineralization		1 (33%)	1 (100%)
Mesenteric artery, thrombosis	1 (33%)	1 (33%)	
Heart	(50)	(48)	(50)
Cardiomyopathy	35 (70%)	40 (83%)	36 (72%)
Inflammation, focal	1 (2%)		1 (2%)
Atrium, thrombosis	5 (10%)	8 (17%)	5 (10%)
Myocardium, embolus		2 (4%)	
Myocardium, inflammation, focal		1 (2%)	
Myocardium, mineralization	1 (2%)	4 (8%)	3 (6%)
Endocrine System			
Adrenal gland, cortex	(50)	(47)	(49)
Degeneration, cystic	1 (2%)		
Degeneration, fatty	3 (6%)		
Degeneration, focal	1 (2%)	1 (2%)	
Hyperplasia, diffuse		1 (2%)	1 (2%)
Hyperplasia, focal	9 (18%)	12 (26%)	13 (27%)
Necrosis			2 (4%)
Necrosis, focal	1 (2%)	1 (2%)	
Pigmentation, hemosiderin	1 (2%)		
Adrenal gland, medulla	(48)	(47)	(49)
Cyst	1 (2%)		
Hyperplasia	20 (42%)	18 (38%)	14 (29%)
Bilateral, hyperplasia	2 (4%)	2 (4%)	2 (4%)
Parathyroid gland	(43)	(42)	(47)
Hyperplasia	3 (7%)	4 (10%)	2 (4%)
Bilateral, hyperplasia	1 (2%)		

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Lesions in Female Rats

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TABLE B4

Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Lifetime Inhalation Study of Talc
(continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Endocrine System (continued)			
Pituitary gland	(50)	(47)	(50)
Cyst	2 (4%)		1 (2%)
Pars distalis, hyperplasia	10 (20%)	6 (13%)	4 (8%)
Pars distalis, necrosis			1 (2%)
Thyroid gland	(50)	(47)	(49)
C-cell, hyperplasia	10 (20%)	8 (17%)	4 (8%)
General Body System			
None			
Genital System			
Clitoral gland	(47)	(44)	(46)
Hyperplasia	2 (4%)		1 (2%)
Inflammation	1 (2%)	1 (2%)	1 (2%)
Ovary	(50)	(47)	(50)
Cyst	5 (10%)		1 (2%)
Uterus	(50)	(48)	(50)
Cyst	1 (2%)	1 (2%)	1 (2%)
Inflammation	1 (2%)	1 (2%)	
Endometrium, hyperplasia	3 (6%)		
Lamina propria, fibrosis	20 (40%)	39 (81%)	19 (38%)
Hematopoietic System			
Bone marrow	(50)	(43)	(49)
Atrophy	1 (2%)	2 (5%)	1 (2%)
Hyperplasia, histiocytic	1 (2%)		1 (2%)
Inflammation, granulomatous, focal	1 (2%)		
Myelofibrosis	1 (2%)	3 (7%)	3 (6%)
Necrosis, focal		1 (2%)	
Myeloid cell, hyperplasia	2 (4%)	2 (5%)	3 (6%)
Lymph node	(50)	(48)	(50)
Axillary, hemorrhage, chronic			1 (2%)
Lymph node, bronchial	(46)	(47)	(47)
Cyst	1 (2%)		
Fibrosis		1 (2%)	
Hemorrhage, chronic		1 (2%)	
Hyperplasia, histiocytic		40 (85%)	45 (96%)
Inflammation, suppurative	1 (2%)		
Pigmentation, hemosiderin	1 (2%)		
Lymph node, mandibular	(47)	(46)	(47)
Hyperplasia, lymphoid		1 (2%)	1 (2%)
Hyperplasia, plasma cell	2 (4%)	1 (2%)	1 (2%)
Inflammation, chronic active	1 (2%)		1 (2%)
Inflammation, suppurative			1 (2%)
Lymph node, mediastinal	(47)	(44)	(47)
Hemorrhage, chronic	1 (2%)		
Hyperplasia, histiocytic		33 (75%)	40 (85%)
Hyperplasia, lymphoid	1 (2%)		1 (2%)
Inflammation, chronic active			1 (2%)
Inflammation, suppurative	1 (2%)	1 (2%)	

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Talc, NTP TR 421

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Lifetime Inhalation Study of Talc
 (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Hematopoietic System (continued)			
Lymph node, mesenteric	(49)	(47)	(47)
Hemorrhage		1 (2%)	
Hyperplasia, lymphoid	2 (4%)	1 (2%)	2 (4%)
Hyperplasia, plasma cell	1 (2%)		
Inflammation, chronic active	4 (8%)	1 (2%)	
Inflammation, granulomatous			1 (2%)
Spleen	(50)	(48)	(50)
Atrophy	2 (4%)	2 (4%)	2 (4%)
Fibrosis, focal	3 (6%)	1 (2%)	1 (2%)
Hematopoietic cell proliferation	4 (8%)	6 (13%)	7 (14%)
Hyperplasia, lymphoid			1 (2%)
Inflammation, granulomatous, focal	1 (2%)		
Pigmentation, hemosiderin	2 (4%)		
Capsule, hemorrhage			1 (2%)
Thymus	(47)	(44)	(47)
Inflammation	1 (2%)		
Integumentary System			
Mammary gland	(50)	(48)	(50)
Galactocoele		1 (2%)	
Hyperplasia, cystic		2 (4%)	
Lobules, hyperplasia			1 (2%)
Skin	(50)	(49)	(50)
Inflammation, focal	1 (2%)		
Musculoskeletal System			
Bone	(50)	(48)	(50)
Fibrous osteodystrophy	4 (8%)	3 (6%)	4 (8%)
Hyperostosis	4 (8%)	1 (2%)	3 (6%)
Pelvis, fracture	1 (2%)		
Vertebra, cyst	1 (2%)		
Nervous System			
Brain	(50)	(48)	(50)
Compression	8 (16%)	7 (15%)	9 (18%)
Hemorrhage		1 (2%)	1 (2%)
Hydrocephalus			1 (2%)
Inflammation, focal		1 (2%)	
White matter, necrosis, focal			2 (4%)
Respiratory System			
Larynx	(50)	(48)	(48)
Inflammation, necrotizing			1 (2%)
Inflammation, suppurative	2 (4%)	1 (2%)	1 (2%)
Lung	(50)	(48)	(50)
Crystals, focal	1 (2%)		
Cyst		1 (2%)	5 (10%)
Cyst, multiple			2 (4%)
Edema	1 (2%)		
Hemorrhage		1 (2%)	1 (2%)
Hyperplasia, adenomatous, diffuse			2 (4%)

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Lesions in Female Rats

B-33

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Lifetime Inhalation Study of Talc
 (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Respiratory System (continued)			
Lung (continued)			
Inflammation, granulomatous	2 (4%)	47 (98%)	50 (100%)
Inflammation, suppurative	2 (4%)	1 (2%)	
Mineralization		2 (4%)	
Alveolar epithelium, hyperplasia	2 (4%)	27 (56%)	47 (94%)
Alveolus, metaplasia, squamous			8 (16%)
Bronchus, epithelium, degeneration, focal	1 (2%)		
Interstitial, fibrosis			1 (2%)
Interstitial, fibrosis, focal	1 (2%)	24 (50%)	44 (88%)
Interstitial, mineralization		1 (2%)	1 (2%)
Peribronchial, hyperplasia, histiocytic		8 (17%)	9 (18%)
Nose	(48)	(45)	(48)
Inflammation, suppurative		1 (2%)	
Lumen, foreign body			1 (2%)
Mucosa, inflammation, suppurative		3 (7%)	5 (10%)
Nasolacrimal duct, inflammation, suppurative	1 (2%)		
Nerve, developmental malformation	1 (2%)		
Olfactory epithelium, metaplasia		1 (2%)	
Respiratory epithelium, hyperplasia	1 (2%)	1 (2%)	2 (4%)
Respiratory epithelium, metaplasia, squamous		1 (2%)	
Trachea	(50)	(48)	(50)
Inflammation, necrotizing			1 (2%)
Inflammation, suppurative	3 (6%)	1 (2%)	2 (4%)
Special Senses System			
Eye	(2)		(2)
Cataract	2 (100%)		2 (100%)
Retina, degeneration	2 (100%)		2 (100%)
Harderian gland	(5)	(7)	(15)
Inflammation	4 (80%)	3 (43%)	3 (20%)
Urinary System			
Kidney	(49)	(47)	(49)
Abscess	1 (2%)		
Cyst		1 (2%)	1 (2%)
Cyst, multiple	1 (2%)		
Embolus, multiple		1 (2%)	
Infarct	1 (2%)		
Infarct, multiple			1 (2%)
Inflammation	1 (2%)	1 (2%)	
Nephropathy	44 (90%)	43 (91%)	42 (86%)
Capsule, inflammation		1 (2%)	
Medulla, inflammation		1 (2%)	1 (2%)
Renal tubule, necrosis	1 (2%)		2 (4%)
Urinary bladder	(50)	(45)	(50)
Inflammation			1 (2%)

^a Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

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C-1

APPENDIX C SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR INHALATION STUDY OF TALC

TABLE C1	Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Talc	C-3
TABLE C2	Individual Animal Tumor Pathology of Male Mice in the 2-Year Inhalation Study of Talc	C-6
TABLE C3	Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Talc	C-24
TABLE C4	Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Talc	C-28

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C-2

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Lesions in Male Mice

C-3

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Talc^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Moribund	1	2	3
Natural deaths	16	18	14
Survivors			
Terminal sacrifice	30	28	32
Missed	1	1	0
Missing	2	1	1
Animals examined microscopically	46	47	49
Alimentary System			
Gallbladder	(31)	(29)	(35)
Intestine large, colon	(36)	(38)	(39)
Intestine small, duodenum	(32)	(30)	(34)
Intestine small, ileum	(33)	(32)	(35)
Adenocarcinoma		1 (3%)	
Liver	(45)	(47)	(48)
Hemangiosarcoma	1 (2%)		1 (2%)
Hemangiosarcoma, metastatic, spleen	1 (2%)		
Hepatocellular carcinoma	6 (13%)	5 (11%)	11 (23%)
Hepatocellular adenoma	1 (2%)	8 (17%)	4 (8%)
Hepatocellular adenoma, multiple	2 (4%)	1 (2%)	
Pancreas	(42)	(39)	(42)
Hepatocellular carcinoma, metastatic, liver	1 (2%)		
Salivary glands	(45)	(46)	(47)
Stomach, glandular	(39)	(43)	(43)
Cardiovascular System			
Heart	(45)	(46)	(49)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Endocrine System			
Adrenal gland	(43)	(46)	(47)
Spindle cell, adenoma	1 (2%)	1 (2%)	1 (2%)
Adrenal gland, cortex	(43)	(46)	(47)
Adenoma		1 (2%)	1 (2%)
Adrenal gland, medulla	(39)	(39)	(42)
Pheochromocytoma malignant	1 (3%)		
Pituitary gland	(44)	(44)	(46)
Adenoma	1 (2%)		
Pars intermedia, adenoma		2 (5%)	
Thyroid gland	(45)	(46)	(45)
Follicular cell, adenoma			2 (4%)
General Body System			
Tissue NOS		(3)	(2)
Hemangioma			1 (50%)
Hemangiosarcoma, metastatic, spleen			1 (50%)

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C-4

Talc, NTP TR 421

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Genital System			
Epididymis	(39)	(39)	(42)
Prostate	(40)	(43)	(44)
Seminal vesicle	(41)	(43)	(39)
Testes	(43)	(44)	(45)
Hemangiosarcoma	1 (2%)		
Hematopoietic System			
Bone marrow	(40)	(42)	(43)
Hemangiosarcoma, metastatic, spleen	1 (3%)		
Lymph node	(45)	(46)	(48)
Lymph node, bronchial	(32)	(39)	(44)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Lymph node, mandibular	(23)	(23)	(19)
Hemangiosarcoma, metastatic, spleen			1 (5%)
Lymph node, mediastinal	(9)	(10)	(7)
Lymph node, mesenteric	(36)	(39)	(40)
Hemangiosarcoma, metastatic, spleen			1 (3%)
Spleen	(44)	(44)	(47)
Hemangiosarcoma	2 (5%)		2 (4%)
Thymus	(34)	(33)	(40)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (3%)
Integumentary System			
None			
Musculoskeletal System			
Bone	(46)	(47)	(49)
Hemangiosarcoma, metastatic, spleen			1 (2%)
Skeletal muscle			(1)
Thoracic, alveolar/bronchiolar carcinoma, metastatic, lung			1 (100%)
Nervous System			
None			
Respiratory System			
Lung	(45)	(47)	(48)
Alveolar/bronchiolar adenoma	6 (13%)	4 (9%)	7 (15%)
Alveolar/bronchiolar adenoma, multiple			2 (4%)
Alveolar/bronchiolar carcinoma	6 (13%)	2 (4%)	2 (4%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)		
Hemangiosarcoma, metastatic, liver	1 (2%)		
Hemangiosarcoma, metastatic, spleen			1 (2%)
Hepatocellular carcinoma, metastatic, liver		1 (2%)	2 (4%)
Special Senses System			
Harderian gland	(1)		(4)
Adenoma	1 (100%)		4 (100%)

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Lesions in Male Mice

C-5

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Urinary System			
Kidney	(45)	(46)	(48)
Carcinoma, metastatic, uncertain primary site	1 (2%)		
Urinary bladder	(43)	(38)	(43)
Sarcoma	1 (2%)		
Systemic Lesions			
Multiple organs ^b	(46)	(47)	(49)
Lymphoma malignant lymphocytic		1 (2%)	
Lymphoma malignant mixed	2 (4%)		
Lymphoma malignant undifferentiated cell	3 (7%)		
Tumor Summary			
Total animals with primary neoplasms ^c	26	20	28
Total primary neoplasms	36	26	38
Total animals with benign neoplasms	11	16	18
Total benign neoplasms	12	17	22
Total animals with malignant neoplasms	20	8	15
Total malignant neoplasms	24	9	16
Total animals with metastatic neoplasms	4	1	4
Total metastatic neoplasms	5	1	11
Total animals with malignant neoplasms, uncertain primary site	1		

^a Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

^b Number of animals with any tissue examined microscopically

^c Primary tumors: all tumors except metastatic tumors

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C-6

Talc, NTP TR 421

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Inhalation Study of Talc: 0 mg/m³

Number of Days on Study	0 4 4 4 4 5 5 5 5 5 5 5 6 6 6 7 7 7 7 7 7
	0 3 3 8 8 1 4 7 7 8 8 8 2 7 8 1 3 3 3 3 3
	8 2 7 4 6 8 3 1 9 5 7 7 9 7 4 0 6 6 6 6 6
Carcass ID Number	4 3 4 5 3 3 4 4 5 3 4 5 5 4 4 5 3 3 3 3 3
	3 9 5 1 7 7 9 0 1 9 8 1 2 9 5 1 6 6 6 7 7
	5 1 4 2 4 2 4 5 5 5 9 8 3 3 5 7 1 4 7 0 1
	1 1
Alimentary System	
Esophagus	M M + M + + + + M + M + + + + + + + +
Gallbladder	M M M M A A A M + + A A + + A M + M + + +
Intestine large	A + A A A A A A + + A + + + A + + + + +
Intestine large, cecum	A + A A A A A A + + A A A + + A + + + + +
Intestine large, colon	A + A A A A A A + + A + + + A + + + + +
Intestine large, rectum	A + A A A A A A + + A A M M + A + + + + +
Intestine small	A + A A A A A A + + A A A + + A + + + + +
Intestine small, duodenum	A A A A A A A A + + A A A + + A + + + + +
Intestine small, ileum	A + A A A A A A + + A A A A + A + + + + +
Intestine small, jejunum	A + A A A A A A + + A A A A + A + + + + +
Liver	A + + + + + + + + + + + + + + + + + + +
Hemangiosarcoma	
Hemangiosarcoma, metastatic, spleen	
Hepatocellular carcinoma	X X X X X X X
Hepatocellular adenoma	
Hepatocellular adenoma, multiple	
Pancreas	M + + + + A + A + + + + + + + A + + + + +
Hepatocellular carcinoma, metastatic, liver	
Salivary glands	A + + + + + + + + + + + + + + + + + + +
Stomach	A + + + + + + + + + + + + + + + + + + +
Stomach, forestomach	A + + + + + + + + I + + M + + + + + + + +
Stomach, glandular	A + A + A M + A + + + A + + + + + + + + +
Cardiovascular System	
Heart	A + + + + + + + + + + + + + + + + + + +
Endocrine System	
Adrenal gland	A + + + M + + + + + + + + + + I + + + + +
Spindle cell, adenoma	
Adrenal gland, cortex	A + + + M + + + + + + + + + + I + + + + +
Adrenal gland, medulla	A + + + M M + + + + + + I + + + M + + + + M
Pheochromocytoma malignant	
Islets, pancreatic	M + I + + M + A + M + A + I M I + + + + + M
Parathyroid gland	M M M + M M M + + + + + M + + + M M M + M
Pituitary gland	M + + + + + + + + + + + + + + + + + + +
Adenoma	
Thyroid gland	A + + + + + + + + + + + + + + + + + + +

+ : Tissue examined microscopically
A: Autolysis precludes examination

M: Missing tissue
I: Insufficient tissue

X: Lesion present
Blank: Not examined

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C-8

Talc, NTP TR 421

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Inhalation Study of Talc: 0 mg/m³ (continued)

Number of Days on Study	0 4 4 4 4 5 5 5 5 5 5 5 6 6 6 7 7 7 7 7 7 7
	0 3 3 8 8 1 4 7 7 8 8 8 2 7 8 1 3 3 3 3 3 3
	8 2 7 4 6 8 3 1 9 5 7 7 9 7 4 0 6 6 6 6 6 6
Carcass ID Number	4 3 4 5 3 3 4 4 5 3 4 5 5 4 4 5 3 3 3 3 3 3
	3 9 5 1 7 7 9 0 1 9 8 1 2 9 5 1 6 6 6 7 7 7
	5 1 4 2 4 2 4 5 5 5 9 8 3 3 5 7 1 4 7 0 1 5
	1 1
General Body System	
None	
Genital System	
Epididymis	+ + + M + + + + + + + + + M + + M + + M I
Preputial gland	+ + + + + + + + + + + + + + + + + + + +
Prostate	M + M M + I + + + + + + + + + I + + + + + + +
Seminal vesicle	+ + + A + M + + + + + A A + + A + + + + + +
Testes	A + + M + + + A + + + + + + + + + + + + + +
Hemangiosarcoma	
Hematopoietic System	
Bone marrow	A + A + + A + A + A + A + + + + + + + + +
Hemangiosarcoma, metastatic, spleen	
Lymph node	M +
Lymph node, bronchial	M M + I + + M + + + + I I + + + + + + + +
Lymph node, mandibular	M M M M + M + M M M M M M + M M M + M M + +
Lymph node, mediastinal	M M M M I M + M M + M M M M M M M M M M +
Lymph node, mesenteric	M + A M + M + M + + M A + + + + + + + + I M
Spleen	A +
Hemangiosarcoma	
Thymus	M I M M + M M A + M + + + I + + M + + + + +
Integumentary System	
Mammary gland	M M M M M M M + M + M I M M M M M M M M M
Skin	+ + + + + + + + + + + + + + + M + + + + + + +
Musculoskeletal System	
Bone	+ + + + + + + + + + + + + + + + + + + +
Nervous System	
Brain	+ + + + + + + + + + + + + + + + + + + +

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C-10

Talc, NTP TR 421

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Inhalation Study of Talc: 0 mg/m³ (continued)

Number of Days on Study	0 4 4 4 4 5 5 5 5 5 5 5 6 6 6 7 7 7 7 7 7 7
	0 3 3 8 8 1 4 7 7 8 8 8 2 7 8 1 3 3 3 3 3 3
	8 2 7 4 6 8 3 1 9 5 7 7 9 7 4 0 6 6 6 6 6 6
Carcass ID Number	4 3 4 5 3 3 4 4 5 3 4 5 5 4 4 5 3 3 3 3 3 3
	3 9 5 1 7 7 9 0 1 9 8 1 2 9 5 1 6 6 6 7 7 7
	5 1 4 2 4 2 4 5 5 5 9 8 3 3 5 7 1 4 7 0 1 5
	1 1
Respiratory System	
Larynx	A + + + + + A + + + + + + + + + I + +
Lung	A + + + + + + + + + + + + + + + + + +
Alveolar/bronchiolar adenoma	
Alveolar/bronchiolar carcinoma	
Alveolar/bronchiolar carcinoma, multiple	
Hemangiosarcoma, metastatic, liver	
Nose	+ + + + + + + A + + + + + + + + + + + +
Trachea	A + A + + + + + + + + + + + A + + + + + +
Special Senses System	
Ear	
Harderian gland	
Adenoma	
Urinary System	
Kidney	A + + + + + + + + + + + + + + + + + +
Carcinoma, metastatic, uncertain primary site	
Urinary bladder	A + + A + + + A + + + + + + + + + + + +
Sarcoma	
Systemic Lesions	
Multiple organs	+ + + + + + + + + + + + + + + + + +
Lymphoma malignant mixed cell type	
Lymphoma malignant undifferentiated cell type	

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C-12

Talc, NTP TR 421

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Inhalation Study of Talc: 6 mg/m³

Number of Days on Study	2	2	3	4	5	5	5	5	5	5	6	6	6	6	6	6	7	7	7	7	7	7	7	7
	5	5	4	2	4	5	5	8	9	9	2	2	3	8	8	8	1	1	2	3	3	3	3	3
	3	3	4	3	6	0	8	4	0	1	4	6	3	1	5	8	0	9	2	6	6	6	6	6
Carcass ID Number	0	1	0	0	1	1	0	1	1	1	0	0	0	1	1	0	0	1	1	0	0	0	0	0
	3	3	4	0	2	6	4	5	5	2	4	7	3	5	0	6	1	5	3	0	0	0	0	0
	5	3	2	7	1	4	0	1	6	9	1	4	8	7	0	1	5	4	2	1	4	5	8	8
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Alimentary System																								
Esophagus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	M	I	M	A	A	A	A	+	A	+	I	A	A	A	+	A	A	M	+	+	+	+	+
Intestine large	A	+	+	A	A	A	A	A	+	A	+	+	A	+	A	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	A	+	A	A	A	A	A	+	A	+	A	A	+	A	+	A	+	+	+	+	+	+	+
Intestine large, colon	A	+	+	A	A	A	A	A	+	A	+	+	A	+	A	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	A	A	A	M	A	+	A	+	A	A	+	A	+	+	M	+	+	+	+	+	+
Intestine small	A	A	+	A	A	A	A	A	+	A	+	A	A	+	A	+	A	+	A	+	+	+	+	+
Intestine small, duodenum	A	A	+	A	A	A	A	A	A	+	A	A	A	+	A	+	A	+	A	+	+	+	+	M
Intestine small, ileum	A	A	A	A	A	A	A	A	+	A	+	A	A	A	+	A	+	A	+	+	+	+	+	+
Adenocarcinoma																								
Intestine small, jejunum	A	A	+	A	A	A	A	+	A	+	A	+	A	+	A	+	A	A	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma					X	X						X				X								
Hepatocellular adenoma												X				X							X	
Hepatocellular adenoma, multiple																								
Pancreas	M	A	+	A	M	A	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	A	+	A	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	A	+	A	A	+	+	+	+	I	A	+	+	+	+	I	+	+	+	+	+	+
Stomach, glandular	+	+	+	A	+	A	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Tooth	+																							
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spindle cell, adenoma																								
Adrenal gland, cortex	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Adrenal gland, medulla	A	+	+	+	+	+	+	+	+	+	+	I	+	+	M	+	+	+	+	M	+	+	+	+
Islets, pancreatic	M	A	M	A	M	A	M	M	+	+	+	+	M	+	I	M	+	+	+	+	M	+	M	M
Parathyroid gland	M	M	M	+	+	I	M	M	M	M	+	+	M	M	+	+	+	+	+	M	M	+	M	M
Pituitary gland	+	+	I	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars intermedia, adenoma																X								
Thyroid gland	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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C-14

Talc, NTP TR 421

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Inhalation Study of Talc: 6 mg/m³ (continued)

Number of Days on Study	2 2 3 4 5 5 5 5 5 6 6 6 6 6 6 7 7 7 7 7 7
	5 5 4 2 4 5 5 8 9 9 2 2 3 8 8 8 1 1 2 3 3 3
	3 3 4 3 6 0 8 4 0 1 4 6 3 1 5 8 0 9 2 6 6 6
Carcass ID Number	0 1 0 0 1 1 0 1 1 1 0 0 0 1 1 0 0 1 1 0 0 0
	3 3 4 0 2 6 4 5 5 2 4 7 3 5 0 6 1 5 3 0 0 0
	5 3 2 7 1 4 0 1 6 9 1 4 8 7 0 1 5 4 2 1 4 5
	1 1
General Body System Tissue NOS	+ + +
Genital System	
Epididymis	A + + + + + + + + + + + + A + + + + + + +
Preputial gland	+ + + + + + + + + + + + + + + + +
Prostate	+ + M A I A + + + + + + + + + + + + +
Seminal vesicle	A + + A + A + + + + + + + A + + + + + + +
Testes	A + + + + A + + + + + + + A + + + + + + +
Hematopoietic System	
Bone marrow	+ + + + A A + A A A + + + + + + + + + +
Lymph node	+ + + + + + + + + + + + + + + + + M +
Lymph node, bronchial	+ + + + M + I + + + + + + + M + + + + M +
Lymph node, mandibular	M M + M M + + + + M + M M M + M + + + M M M
Lymph node, mediastinal	M M M M M + M M M + M M M M M M M + + M M M
Lymph node, mesenteric	M M M M + M + + + + + + M + + + M + + + M +
Spleen	A + + + + A A + + + + + + + + + + + + +
Thymus	A M M M + + + I + + M + M + I M + + + + + + +
Integumentary System	
Mammary gland	M M M M M A M M M M M M M M + M + + M + M M
Skin	+ +
Musculoskeletal System	
Bone	+ +
Nervous System	
Brain	+ +
Respiratory System	
Larynx	A + + A + A A + + + + + A + + + + + + I + +
Lung	+ +
Alveolar/bronchiolar adenoma	
Alveolar/bronchiolar carcinoma	X
Hepatocellular carcinoma, metastatic, liver	X
Nose	+ + + + + A + + + + + + + + + + + + + + +
Trachea	+ + + + + A I + + + + + A + + + + + + + + + +

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[illegible]

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Talc, NTP TR 421

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Inhalation Study of Talc: 6 mg/m³ (continued)

Number of Days on Study	2	2	3	4	5	5	5	5	5	5	6	6	6	6	6	7	7	7	7	7	7
	5	5	4	2	4	5	5	8	9	9	2	2	3	8	8	8	1	1	2	3	3
	3	3	4	3	6	0	8	4	0	1	4	6	3	1	5	8	0	9	2	6	6
Carcass ID Number	0	1	0	0	1	1	0	1	1	1	0	0	0	1	1	0	0	1	1	0	0
	3	3	4	0	2	6	4	5	5	2	4	7	3	5	0	6	1	5	3	0	0
	5	3	2	7	1	4	0	1	6	9	1	4	8	7	0	1	5	4	2	1	4
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Special Senses System	None																				
Urinary System																					
Kidney	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	A	+	+	A	+	A	A	+	+	+	+	A	+	A	+	A	+	+	+	+	+
Systemic Lesions																					
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																				X	

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Individual Animal Tumor Pathology of Non-Tumor			
Number of Days on Study	7 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 4 4 4 4 4 4 6 6 7 7 7 7 7 7 7 7 8 8 8 8 8 8 9 9 0 0 0 0 0 0		
Carcass ID Number	0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 0 0 1 1 1 1 1 1 3 3 6 6 6 7 7 9 9 9 9 0 0 2 3 5 3 9 2 2 5 6 0 4 3 6 4 5 6 1 2 3 5 6 9 1 5 7 5 5 2 2 4 5 8 2 1	Total Tissues/ Tumors	
Special Senses System	None		
Urinary System			
Kidney	+	+	46
Urinary bladder	+	+	38
Systemic Lesions			
Multiple organs	+	+	47
Lymphoma malignant lymphocytic			1

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[illegible]

Pltf JNJ 00000929

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Talc, NTP TR 421

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Inhalation Study of Talc: 18 mg/m³ (continued)

Number of Days on Study	0 1 1 1 4 4 4 5 5 5 6 6 7 7 7 7 7 7 7 7 7
	2 1 1 5 2 3 5 7 3 4 5 5 7 2 2 2 3 3 3 3 3
	8 4 5 9 2 8 7 8 8 1 4 8 2 1 4 5 7 6 6 6 6
Carcass ID Number	1 1 1 3 2 1 2 2 1 2 2 2 2 2 3 3 1 1 1 2 2
	8 8 9 1 8 8 2 1 8 7 4 8 4 8 1 0 4 8 8 9 1
	7 3 5 0 5 6 1 9 5 7 9 3 1 2 5 6 3 4 9 2 3
	1 1
Genital System	
Epididymis	+ + + + A + + + + M + + A + + + + + M + + +
Preputial gland	+ + + + +
Prostate	+ M M M A + + + + + A + + + + + + + + + +
Seminal vesicle	+ M M M A + + + + + A A M A + + + + + + + +
Testes	+ + + + A + + + + + A M + A + + + + + + + +
Hematopoietic System	
Bone marrow	+ + + + A A + + A A A A + + + + + + + + +
Lymph node	+ + + + + + + + + M + + + + + + + + + +
Lymph node, bronchial	+ + M M A + + + + + M + + + + + + + + + +
Alveolar/bronchiolar carcinoma, metastatic, lung	X
Lymph node, mandibular	M I I + A M + M + M M M M + + + + + M M M M
Hemangiosarcoma, metastatic, spleen	X
Lymph node, mediastinal	M M M M M M M M M M M + M + M M + M M M M M
Lymph node, mesenteric	M + + M A + + M + + A + + A + + M + + + + +
Hemangiosarcoma, metastatic, spleen	X
Spleen	+ + + + A + + + + + A + + + + + + + + + +
Hemangiosarcoma	X X
Thymus	+ M + M M + + + + + A + M + + + + + + M + + +
Alveolar/bronchiolar carcinoma, metastatic, lung	X
Integumentary System	
Mammary gland	I I M M M M M + + M M M M M M M M M M M M
Skin	+ + + + + + + + + + + + + + + + + + + +
Musculoskeletal System	
Bone	+ + + + + + + + + + + + + + + + + + + +
Hemangiosarcoma, metastatic, spleen	X
Skeletal muscle	+
Thoracic, alveolar/bronchiolar carcinoma, metastatic, lung	X
Nervous System	
Brain	+ + + + A + + + + + + + + + + + + + + +

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TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Inhalation Study of Talc: 18 mg/m³ (continued)

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JNJ 000009097

Talc, NTP TR 421

C-24

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Talc

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Harderian Gland: Adenoma			
Overall rates ^a	1/46 (2%)	0/47 (0%)	4/49 (8%)
Adjusted rates ^b	3.3%	0.0%	12.0%
Terminal rates ^c	1/30 (3%)	0/28 (0%)	3/32 (9%)
First incidence (days)	736 (T)	- ^e	725
Life table tests ^d	P=0.073	P=0.514N	P=0.204
Logistic regression tests ^d	P=0.075	P=0.514N	P=0.216
Cochran-Armitage test ^d	P=0.065		
Fisher exact test ^d		P=0.495N	P=0.201
Liver: Hepatocellular Adenoma			
Overall rates	3/45 (7%)	9/47 (19%)	4/48 (8%)
Adjusted rates	10.0%	29.5%	11.8%
Terminal rates	3/30 (10%)	7/28 (25%)	3/32 (9%)
First incidence (days)	736 (T)	633	672
Life table tests	P=0.489N	P=0.050	P=0.539
Logistic regression tests	P=0.493N	P=0.061	P=0.552
Cochran-Armitage test	P=0.515N		
Fisher exact test		P=0.070	P=0.536
Liver: Hepatocellular Carcinoma			
Overall rates	6/45 (13%)	5/47 (11%)	11/48 (23%)
Adjusted rates	16.7%	13.7%	27.3%
Terminal rates	2/30 (7%)	1/28 (4%)	5/32 (16%)
First incidence (days)	571	546	438
Life table tests	P=0.114	P=0.491N	P=0.187
Logistic regression tests	P=0.116	P=0.445N	P=0.203
Cochran-Armitage test	P=0.097		
Fisher exact test		P=0.469N	P=0.177
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rates	9/45 (20%)	13/47 (28%)	14/48 (29%)
Adjusted rates	25.6%	38.1%	34.5%
Terminal rates	5/30 (17%)	8/28 (29%)	7/32 (22%)
First incidence (days)	571	546	438
Life table tests	P=0.256	P=0.228	P=0.230
Logistic regression tests	P=0.216	P=0.257	P=0.223
Cochran-Armitage test	P=0.225		
Fisher exact test		P=0.269	P=0.217
Lung: Alveolar/bronchiolar Adenoma			
Overall rates	6/45 (13%)	4/47 (9%)	9/48 (19%)
Adjusted rates	20.0%	14.3%	27.0%
Terminal rates	6/30 (20%)	4/28 (14%)	8/32 (25%)
First incidence (days)	736 (T)	736 (T)	672
Life table tests	P=0.224	P=0.411N	P=0.333
Logistic regression tests	P=0.251	P=0.411N	P=0.371
Cochran-Armitage test	P=0.210		
Fisher exact test		P=0.342N	P=0.336

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Lesions in Male Mice

C-25

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Lung: Alveolar/bronchiolar Carcinoma			
Overall rates	7/45 (16%)	2/47 (4%)	2/48 (4%)
Adjusted rates	23.3%	5.9%	5.2%
Terminal rates	7/30 (23%)	1/28 (4%)	0/32 (0%)
First incidence (days)	736 (T)	558	438
Life table tests	P=0.068N	P=0.093N	P=0.068N
Logistic regression tests	P=0.069N	P=0.073N	P=0.070N
Cochran-Armitage test	P=0.065N		
Fisher exact test		P=0.069N	P=0.065N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rates	12/45 (27%)	5/47 (11%)	11/48 (23%)
Adjusted rates	40.0%	16.4%	30.8%
Terminal rates	12/30 (40%)	4/28 (14%)	8/32 (25%)
First incidence (days)	736 (T)	558	438
Life table tests	P=0.533N	P=0.063N	P=0.426N
Logistic regression tests	P=0.552N	P=0.043N	P=0.423N
Cochran-Armitage test	P=0.554N		
Fisher exact test		P=0.043N	P=0.429N
Pituitary Gland (Pars Intermedia): Adenoma			
Overall rates	0/44 (0%)	2/44 (5%)	0/46 (0%)
Adjusted rates	0.0%	6.5%	0.0%
Terminal rates	0/29 (0%)	1/27 (4%)	0/32 (0%)
First incidence (days)	-	681	- ^f
Life table tests	P=0.547N	P=0.238	-
Logistic regression tests	P=0.566N	P=0.239	-
Cochran-Armitage test	P=0.564N		
Fisher exact test		P=0.247	-
Spleen: Hemangiosarcoma			
Overall rates	2/44 (5%)	0/44 (0%)	2/47 (4%)
Adjusted rates	6.9%	0.0%	5.5%
Terminal rates	2/29 (7%)	0/28 (0%)	0/32 (0%)
First incidence (days)	736 (T)	-	672
Life table tests	P=0.595	P=0.246N	P=0.650N
Logistic regression tests	P=0.581	P=0.246N	P=0.668N
Cochran-Armitage test	P=0.577		
Fisher exact test		P=0.247N	P=0.666N
All Organs: Hemangiosarcoma			
Overall rates	4/46 (9%)	0/47 (0%)	3/49 (6%)
Adjusted rates	12.9%	0.0%	8.4%
Terminal rates	3/30 (10%)	0/28 (0%)	1/32 (3%)
First incidence (days)	710	-	672
Life table tests	P=0.529N	P=0.071N	P=0.448N
Logistic regression tests	P=0.545N	P=0.060N	P=0.456N
Cochran-Armitage test	P=0.554N		
Fisher exact test		P=0.056N	P=0.464N

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Talc, NTP TR 421

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
All Organs: Hemangioma or Hemangiosarcoma			
Overall rates	4/46 (9%)	0/47 (0%)	4/49 (8%)
Adjusted rates	12.9%	0.0%	11.4%
Terminal rates	3/30 (10%)	0/28 (0%)	2/32 (6%)
First incidence (days)	710	—	672
Life table tests	P=0.515	P=0.071N	P=0.590N
Logistic regression tests	P=0.505	P=0.060N	P=0.598N
Cochran-Armitage test	P=0.492		
Fisher exact test		P=0.056N	P=0.607N
All Organs: Malignant Lymphoma (Lymphocytic, Mixed, or Undifferentiated Cell Type)			
Overall rates	5/46 (11%)	1/47 (2%)	0/49 (0%)
Adjusted rates	16.7%	3.6%	0.0%
Terminal rates	5/30 (17%)	1/28 (4%)	0/32 (0%)
First incidence (days)	736 (T)	736 (T)	—
Life table tests	P=0.019N	P=0.116N	P=0.027N
Logistic regression tests	P=0.019N	P=0.116N	P=0.027N
Cochran-Armitage test	P=0.020N		
Fisher exact test		P=0.097N	P=0.024N
All Organs: Benign Tumors			
Overall rates	11/46 (24%)	16/47 (34%)	18/49 (37%)
Adjusted rates	35.2%	51.1%	51.4%
Terminal rates	10/30 (33%)	13/28 (46%)	15/32 (47%)
First incidence (days)	587	633	672
Life table tests	P=0.158	P=0.135	P=0.127
Logistic regression tests	P=0.154	P=0.188	P=0.138
Cochran-Armitage test	P=0.139		
Fisher exact test		P=0.199	P=0.128
All Organs: Malignant Tumors			
Overall rates	20/46 (43%)	8/47 (17%)	15/49 (31%)
Adjusted rates	58.3%	23.3%	35.9%
Terminal rates	16/30 (53%)	4/28 (14%)	6/32 (19%)
First incidence (days)	571	546	438
Life table tests	P=0.253N	P=0.012N	P=0.166N
Logistic regression tests	P=0.262N	P=0.005N	P=0.152N
Cochran-Armitage test	P=0.245N		
Fisher exact test		P=0.005N	P=0.139N
All Organs: Benign or Malignant Tumors			
Overall rates	26/46 (57%)	20/47 (43%)	28/49 (57%)
Adjusted rates	76.2%	58.0%	66.5%
Terminal rates	22/30 (73%)	14/28 (50%)	18/32 (56%)
First incidence (days)	571	546	438
Life table tests	P=0.442	P=0.208N	P=0.554
Logistic regression tests	P=0.344	P=0.102N	P=0.503
Cochran-Armitage test	P=0.399		
Fisher exact test		P=0.127N	P=0.558

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Lesions in Male Mice

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TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Talc (continued)

- (T) Terminal sacrifice
- ^a Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
- ^b Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
- ^c Observed incidence at terminal kill
- ^d Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
- ^e Not applicable; no tumors in animal group
- ^f Value of statistic cannot be computed.

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C-28

Talc, NTP TR 421

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Talc^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Moribund	1	2	3
Natural deaths	16	18	14
Survivors			
Terminal sacrifice	30	28	32
Missexed	1	1	0
Missing	2	1	1
Animals examined microscopically	46	47	49
Alimentary System			
Gallbladder	(31)	(29)	(35)
Dilatation			1 (3%)
Epithelium, hyperplasia, papillary			1 (3%)
Intestine large, cecum	(34)	(35)	(37)
Hyperplasia, lymphoid		1 (3%)	3 (8%)
Intestine large, colon	(36)	(38)	(39)
Hyperplasia, lymphoid	1 (3%)		
Intestine large, rectum	(32)	(32)	(31)
Serosa, inflammation, suppurative		1 (3%)	
Intestine small, duodenum	(32)	(30)	(34)
Hyperplasia, lymphoid			1 (3%)
Mucosa, atrophy	3 (9%)	7 (23%)	3 (9%)
Intestine small, ileum	(33)	(32)	(35)
Hyperplasia, lymphoid	5 (15%)	3 (9%)	5 (14%)
Mucosa, atrophy	3 (9%)	5 (16%)	4 (11%)
Peyer's patch, necrosis	1 (3%)		
Intestine small, jejunum	(32)	(31)	(36)
Hyperplasia, lymphoid			1 (3%)
Mucosa, atrophy	3 (9%)	3 (10%)	2 (6%)
Liver	(45)	(47)	(48)
Abscess	1 (2%)		1 (2%)
Focal cellular change	4 (9%)	3 (6%)	5 (10%)
Hematocyst		1 (2%)	
Hematopoietic cell proliferation	2 (4%)	2 (4%)	
Infarct	2 (4%)		
Inflammation, focal		3 (6%)	1 (2%)
Mineralization, focal		1 (2%)	
Necrosis, focal	4 (9%)	5 (11%)	4 (8%)
Pigmentation, hemosiderin, focal			1 (2%)
Bile duct, hyperplasia, focal			1 (2%)
Serosa, inflammation, suppurative			1 (2%)
Pancreas	(42)	(39)	(42)
Serosa, inflammation, suppurative			1 (2%)
Stomach, forestomach	(43)	(41)	(46)
Hyperplasia, squamous, focal		1 (2%)	1 (2%)
Tooth		(3)	
Dysplasia		3 (100%)	
Cardiovascular System			
Heart	(45)	(46)	(49)
Thrombosis		1 (2%)	1 (2%)
Coronary artery, mineralization		1 (2%)	
Myocardium, degeneration, focal	1 (2%)		
Myocardium, fibrosis, focal		1 (2%)	

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Lesions in Male Mice

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TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Endocrine System			
Adrenal gland	(43)	(46)	(47)
Spindle cell, hyperplasia	38 (88%)	37 (80%)	35 (74%)
Adrenal gland, cortex	(43)	(46)	(47)
Atrophy	1 (2%)		
Hyperplasia, focal		1 (2%)	
Vacuolization cytoplasmic, focal		3 (7%)	4 (9%)
Parathyroid gland	(25)	(21)	(26)
Cyst	3 (12%)	1 (5%)	
Pituitary gland	(44)	(44)	(46)
Cyst	1 (2%)		1 (2%)
Pigmentation, lipofuscin	1 (2%)		
Thyroid gland	(45)	(46)	(45)
Cyst	2 (4%)	1 (2%)	1 (2%)
Follicular cell, hyperplasia	4 (9%)	8 (17%)	8 (18%)
General Body System			
None			
Genital System			
Epididymis	(39)	(39)	(42)
Inflammation, suppurative	1 (3%)	1 (3%)	
Preputial gland	(8)	(6)	(8)
Dilatation	7 (88%)	6 (100%)	8 (100%)
Inflammation	3 (38%)		1 (13%)
Prostate	(40)	(43)	(44)
Inflammation, suppurative	3 (8%)	7 (16%)	4 (9%)
Epithelium, hyperplasia		1 (2%)	
Seminal vesicle	(41)	(43)	(39)
Inflammation, suppurative		2 (5%)	1 (3%)
Testes	(43)	(44)	(45)
Aspermatogenesis, diffuse			1 (2%)
Atrophy, diffuse			1 (2%)
Hypospermia	1 (2%)	2 (5%)	
Inflammation, suppurative		1 (2%)	
Seminiferous tubule, degeneration, focal	3 (7%)	4 (9%)	1 (2%)
Hematopoietic System			
Bone marrow	(40)	(42)	(43)
Hyperplasia	4 (10%)	1 (2%)	1 (2%)
Myelofibrosis	2 (5%)	2 (5%)	
Myeloid cell, hyperplasia	4 (10%)	7 (17%)	1 (2%)
Lymph node	(45)	(46)	(48)
Iliac, hyperplasia, lymphoid	1 (2%)		
Iliac, hyperplasia, plasma cell	1 (2%)		
Lumbar, hyperplasia, lymphoid	1 (2%)	1 (2%)	
Lumbar, hyperplasia, plasma cell		1 (2%)	
Pancreatic, inflammation, granulomatous		1 (2%)	
Renal, depletion lymphoid			1 (2%)
Renal, hyperplasia, lymphoid			1 (2%)

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TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Hematopoietic System (continued)			
Lymph node, bronchial	(32)	(39)	(44)
Abscess			1 (2%)
Hyperplasia, histiocytic	1 (3%)	32 (82%)	42 (95%)
Hyperplasia, histiocytic, lymphoid		1 (3%)	
Hyperplasia, lymphoid	3 (9%)	10 (26%)	23 (52%)
Infiltration cellular, mixed cell	3 (9%)	1 (3%)	3 (7%)
Inflammation, acute	1 (3%)		
Follicular, necrosis	1 (3%)		
Lymph node, mandibular	(23)	(23)	(19)
Hyperplasia, histiocytic		1 (4%)	1 (5%)
Hyperplasia, lymphoid			1 (5%)
Follicular, necrosis			
Lymph node, mediastinal	(9)	(10)	(7)
Hyperplasia, histiocytic	1 (11%)	1 (10%)	2 (29%)
Hyperplasia, lymphoid		2 (20%)	
Lymph node, mesenteric	(36)	(39)	(40)
Depletion lymphoid	1 (3%)		2 (5%)
Hyperplasia, lymphoid	4 (11%)	3 (8%)	6 (15%)
Infiltration cellular, mixed cell	18 (50%)	20 (51%)	13 (33%)
Inflammation, granulomatous		1 (3%)	
Thrombosis			1 (3%)
Follicular, necrosis		6 (15%)	2 (5%)
Spleen	(44)	(44)	(47)
Hematocyst		1 (2%)	
Hematopoietic cell proliferation	6 (14%)	7 (16%)	10 (21%)
Hyperplasia, lymphoid	3 (7%)	2 (5%)	3 (6%)
Hyperplasia, mast cell			1 (2%)
Inflammation, granulomatous		1 (2%)	
Lymphoid follicle, depletion lymphoid		2 (5%)	5 (11%)
Lymphoid follicle, necrosis	2 (5%)	5 (11%)	1 (2%)
Thymus	(34)	(33)	(40)
Cyst	3 (9%)	2 (6%)	1 (3%)
Hyperplasia, lymphoid			1 (3%)
Inflammation, granulomatous		1 (3%)	
Necrosis	1 (3%)		2 (5%)
Cortex, depletion lymphoid	6 (18%)	10 (30%)	8 (20%)
Epithelial cell, hyperplasia, focal	1 (3%)		
Integumentary System			
Skin	(44)	(45)	(48)
Abscess		1 (2%)	
Alopecia	1 (2%)		1 (2%)
Inflammation, acute		2 (4%)	
Ulcer, focal		2 (4%)	
Musculoskeletal System			
Bone	(46)	(47)	(49)
Rib, cartilage, fracture healed	1 (2%)		
Nervous System			
Brain	(46)	(47)	(48)
Mineralization, focal	37 (80%)	39 (83%)	38 (79%)

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Lesions in Male Mice

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TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Respiratory System			
Larynx	(42)	(41)	(46)
Inflammation, acute	1 (2%)		1 (2%)
Lung	(45)	(47)	(48)
Congestion	3 (7%)	1 (2%)	1 (2%)
Hyperplasia, macrophage	3 (7%)	46 (98%)	48 (100%)
Inflammation, chronic active		16 (34%)	40 (83%)
Thrombosis			1 (2%)
Alveolar epithelium, hyperplasia, focal	1 (2%)		
Peribronchiolar, inflammation, chronic active		1 (2%)	
Perivascular, inflammation, suppurative	1 (2%)		
Nose	(45)	(46)	(47)
Cytoplasmic alteration, focal	5 (11%)	23 (50%)	40 (85%)
Erosion, focal	1 (2%)	1 (2%)	2 (4%)
Inflammation, acute	4 (9%)	4 (9%)	7 (15%)
Special Senses System			
Ear	(1)		
Inflammation, granulomatous	1 (100%)		
Urinary System			
Kidney	(45)	(46)	(48)
Casts protein	1 (2%)		
Cyst	2 (4%)		
Hydronephrosis	3 (7%)	1 (2%)	
Inflammation, suppurative, focal	3 (7%)	5 (11%)	3 (6%)
Metaplasia, osseous, focal		3 (7%)	
Nephropathy, chronic	3 (7%)		2 (4%)
Capsule, inflammation, suppurative			1 (2%)
Pelvis, inflammation, suppurative	2 (4%)	5 (11%)	1 (2%)
Urinary bladder	(43)	(38)	(43)
Dysplasia, focal	1 (2%)		
Inflammation, chronic active	6 (14%)	5 (13%)	2 (5%)
Ulcer, focal			1 (2%)
Transitional epithelium, hyperplasia		1 (3%)	

^a Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

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D-1

APPENDIX D SUMMARY LESIONS IN FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF TALC

TABLE D1	Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Talc	D-3
TABLE D2	Individual Animal Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Talc	D-6
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Lesions in Female Mice

D-3

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Talc^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Moribund	2	4	4
Natural deaths	17	21	21
Survivors			
Terminal sacrifice	30	23	25
Missing	1	1	
Culled		1	
Animals examined microscopically	46	48	50
Alimentary System			
Esophagus	(43)	(47)	(48)
Gallbladder	(31)	(28)	(29)
Intestine large, cecum	(35)	(29)	(34)
Leiomyoma			1 (3%)
Intestine large, colon	(38)	(33)	(32)
Leiomyosarcoma		1 (3%)	
Intestine small, ileum	(33)	(27)	(31)
Liver	(46)	(46)	(50)
Hemangioma		1 (2%)	
Hepatocellular carcinoma	7 (15%)	5 (11%)	4 (8%)
Hepatocellular adenoma	5 (11%)	1 (2%)	4 (8%)
Mesentery	(2)		
Pancreas	(42)	(39)	(44)
Salivary glands	(46)	(48)	(50)
Hemangioma	1 (2%)		
Stomach, glandular	(45)	(39)	(46)
Cardiovascular System			
Heart	(46)	(48)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	
Endocrine System			
Adrenal gland	(46)	(45)	(50)
Spindle cell, adenoma	1 (2%)		
Adrenal gland, cortex	(46)	(44)	(50)
Adenoma	1 (2%)		
Adrenal gland, medulla	(41)	(43)	(45)
Pheochromocytoma malignant	1 (2%)		
Pituitary gland	(42)	(42)	(48)
Adenoma	5 (12%)	4 (10%)	2 (4%)
Carcinoma		2 (5%)	
Thyroid gland	(43)	(47)	(49)
Follicular cell, adenoma	1 (2%)	2 (4%)	2 (4%)

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TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
General Body System			
Tissue NOS	(4)	(1)	(2)
Fibrosarcoma	1 (25%)		
Hemangioma	1 (25%)		1 (50%)
Hemangiosarcoma			1 (50%)
Genital System			
Ovary	(38)	(43)	(46)
Adenocarcinoma, metastatic, uterus	1 (3%)		
Adenoma	1 (3%)	1 (2%)	
Cystadenoma		1 (2%)	
Luteoma	2 (5%)		
Uterus	(44)	(45)	(49)
Adenocarcinoma	1 (2%)		
Carcinoma adenosquamous			1 (2%)
Hematopoietic System			
Bone marrow	(41)	(43)	(45)
Lymph node	(46)	(46)	(49)
Lymph node, bronchial	(38)	(37)	(43)
Adenocarcinoma, metastatic, kidney		1 (3%)	
Adenocarcinoma, metastatic, uterus	1 (3%)		
Alveolar/bronchiolar carcinoma, metastatic, lung		3 (8%)	
Lymph node, mandibular	(35)	(38)	(36)
Lymph node, mediastinal	(13)	(17)	(14)
Adenocarcinoma, metastatic, kidney		1 (6%)	
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (6%)	
Lymph node, mesenteric	(35)	(31)	(37)
Spleen	(45)	(44)	(50)
Hemangiosarcoma			1 (2%)
Thymus	(40)	(40)	(41)
Alveolar/bronchiolar carcinoma, metastatic, lung		2 (5%)	
Integumentary System			
Mammary gland	(41)	(45)	(48)
Fibrosarcoma			1 (2%)
Musculoskeletal System			
Bone	(46)	(48)	(50)
Vertebra, alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	
Nervous System			
Spinal cord			(1)
Thoracic, ganglioneuroma			1 (100%)

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Lesions in Female Mice

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TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Respiratory System			
Lung	(46)	(48)	(50)
Adenocarcinoma, metastatic, kidney		1 (2%)	
Alveolar/bronchiolar adenoma	3 (7%)	2 (4%)	2 (4%)
Alveolar/bronchiolar carcinoma	2 (4%)	4 (8%)	1 (2%)
Hemangiosarcoma, metastatic, tissue NOS			1 (2%)
Hepatocellular carcinoma, metastatic, liver	2 (4%)	2 (4%)	
Trachea	(40)	(36)	(45)
Special Senses System			
Harderian gland	(2)	(2)	(1)
Adenocarcinoma			1 (100%)
Adenoma	2 (100%)	1 (50%)	
Urinary System			
Kidney	(46)	(46)	(50)
Adenocarcinoma		1 (2%)	
Hepatocellular carcinoma, metastatic, liver	1 (2%)		
Urinary bladder	(44)	(40)	(41)
Systemic Lesions			
Multiple organs ^b	(46)	(48)	(50)
Lymphoma malignant histiocytic			1 (2%)
Lymphoma malignant lymphocytic	2 (4%)	3 (6%)	3 (6%)
Lymphoma malignant mixed	3 (7%)	4 (8%)	2 (4%)
Lymphoma malignant undifferentiated cell	2 (4%)		2 (4%)
Tumor Summary			
Total animals with primary neoplasms ^c	31	26	21
Total primary neoplasms	42	33	31
Total animals with benign neoplasms	18	9	10
Total benign neoplasms	23	13	13
Total animals with malignant neoplasms	19	19	15
Total malignant neoplasms	19	20	18
Total animals with metastatic neoplasms	3	5	1
Total metastatic neoplasms	5	13	1

^a Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.^b Number of animals with any tissue examined microscopically^c Primary tumors: all tumors except metastatic tumors

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TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Talc: 0 mg/m³

Number of Days on Study	0	4	4	4	5	5	5	5	5	5	6	6	6	6	6	7	7	7	7	7
	3	2	6	8	0	0	0	4	5	9	4	8	8	8	9	2	2	2	2	2
	0	6	5	7	5	6	9	4	2	8	1	0	3	6	2	3	9	9	9	9
Carcass ID Number	5	5	5	3	4	4	3	4	4	4	5	5	4	5	4	4	3	3	3	3
	3	0	3	7	1	2	8	1	7	7	0	2	9	0	4	9	7	8	8	8
	3	0	4	6	7	0	2	5	5	3	5	8	7	7	6	6	7	1	4	6
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Alimentary System																				
Esophagus	+	+	M	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	M	A	M	A	M	A	+	A	A	A	A	+	A	A	+	+	+	+	+
Intestine large	A	+	+	A	A	A	+	+	A	+	A	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	A	A	A	A	A	A	+	A	+	A	+	A	+	+	+	+	+	+
Intestine large, colon	A	+	A	A	A	A	+	+	A	+	A	+	+	+	+	+	+	M	+	+
Intestine large, rectum	A	M	+	M	M	A	+	+	M	A	A	M	+	+	+	+	+	+	+	M
Intestine small	A	+	A	A	A	A	A	+	A	A	A	A	A	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	A	A	A	A	A	A	A	A	A	A	A	+	+	+	+	+	+	M
Intestine small, ileum	A	+	A	A	A	A	A	A	A	A	A	A	A	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	A	A	A	A	A	+	A	A	A	A	A	+	A	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma			X				X				X		X							X
Hepatocellular adenoma																				X
Mesentery								+												
Pancreas	+	+	A	+	A	A	+	+	+	+	+	I	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																				
Stomach	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																				
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																				
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spindle cell, adenoma	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex																				
Adenoma																				
Adrenal gland, medulla	+	+	+	+	M	+	M	+	+	+	I	I	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																				
Islets, pancreatic	+	+	M	+	A	A	M	+	I	M	I	M	I	M	+	+	+	+	+	+
Parathyroid gland	M	M	+	M	+	+	M	+	M	I	+	M	+	+	+	+	M	M	I	M
Pituitary gland	M	+	+	+	M	M	+	+	+	+	I	+	+	+	+	+	+	+	+	+
Adenoma													X	X	X					X
Thyroid gland	A	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																				

+: Tissue examined microscopically
A: Autolysis precludes examination

M: Missing tissue
I: Insufficient tissue

X: Lesion present
Blank: Not examined

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Talc, NTP TR 421

TABLE D2

Individual Animal Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Talc: 0 mg/m³ (continued)

Number of Days on Study	0 4 4 4 5 5 5 5 5 5 6 6 6 6 7 7 7 7 7 7 3 2 6 8 0 0 0 4 5 9 4 8 8 8 9 2 2 2 2 2 0 6 5 7 5 6 9 4 2 8 1 0 3 6 2 3 9 9 9 9
Carcass ID Number	5 5 5 3 4 4 3 4 4 4 5 5 4 5 4 4 3 3 3 3 3 0 3 7 1 2 8 1 7 7 0 2 9 0 4 9 7 8 8 8 3 0 4 6 7 0 2 5 5 3 5 8 7 7 6 6 7 1 4 6 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
General Body System	
Tissue NOS	
Fibrosarcoma	
Hemangioma	
Genital System	
Ovary	+ + + + + + I + + I M + + + + + + + +
Adenocarcinoma, metastatic, uterus	
Adenoma	
Luteoma	
Uterus	M I + + + + + + + + + + + + + + + +
Adenocarcinoma	
Hematopoietic System	
Bone marrow	+ + A + A A + A A + + + + + + + + + +
Lymph node	+ + + + + + + + + + + + + + + + + +
Lymph node, bronchial	M + + + + M + M M + + + M + + + + + +
Adenocarcinoma, metastatic, uterus	
Lymph node, mandibular	+ + M + + + + + + M + + + M + + + M M +
Lymph node, mediastinal	M M + M M M + M + M M M + M + + M M + M +
Lymph node, mesenteric	M + + M M M + M M + + + + I + + + M + + +
Spleen	+ + + + + A + + + + + + + + + + + +
Thymus	M M + + + M + + + + + + + M + M + + + +
Integumentary System	
Mammary gland	A + + + + A + I + + + + M + + + + + + + +
Skin	+ + + + + + + + + + + + + + + + + +
Musculoskeletal System	
Bone	+ + + + + + + + + + + + + + + + + +
Nervous System	
Brain	+ + + + + + + + + + + + + + + + + +

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D-10

Talc, NTP TR 421

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Talc: 0 mg/m³ (continued)

Number of Days on Study	0 4 4 4 5 5 5 5 5 5 6 6 6 6 6 7 7 7 7 7
	3 2 6 8 0 0 0 4 5 9 4 8 8 8 9 2 2 2 2 2
	0 6 5 7 5 6 9 4 2 8 1 0 3 6 2 3 9 9 9 9
Carcass ID Number	5 5 5 3 4 4 3 4 4 4 5 5 4 5 4 4 3 3 3 3
	3 0 3 7 1 2 8 1 7 7 0 2 9 0 4 9 7 8 8 8
	3 0 4 6 7 0 2 5 5 3 5 8 7 7 6 6 7 1 4 6
	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Respiratory System	
Larynx	+ + + + + A I + + + + + + + + + + +
Lung	+ + + + + + + + + + + + + + + + +
Alveolar/bronchiolar adenoma	
Alveolar/bronchiolar carcinoma	
Hepatocellular carcinoma, metastatic, liver	
Nosc	+ + + + + + + + + + + + + + + + +
Trachea	A + A + M A A + + + + + + + + + + +
Special Senses System	
Harderian gland	
Adenoma	
Urinary System	
Kidney	+ + + + + + + + + + + + + + + + +
Hepatocellular carcinoma, metastatic, liver	
Urinary bladder	M + + + + A + + + + + + + + + + +
Systemic Lesions	
Multiple organs	+ + + + + + + + + + + + + + + + +
Lymphoma malignant lymphocytic	
Lymphoma malignant mixed	
Lymphoma malignant undifferentiated cell type	

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D-12

Talc, NTP TR 421

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Talc: 6 mg/m³

Number of Days on Study	0 0 4 4 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 7 7 7
	2 9 2 9 0 3 4 5 5 6 1 2 2 4 4 6 7 7 8 9 9 0 0 1
	0 2 2 1 0 4 8 4 9 4 8 1 8 1 5 5 6 8 6 2 9 9 9 2
Carcass ID Number	1 0 0 1 0 1 1 1 0 1 0 1 0 0 1 1 0 1 1 0 0 1 1 0
	1 5 1 1 5 4 7 4 1 7 2 2 5 8 3 0 5 7 3 8 6 1 4 2
	7 5 6 5 0 7 6 8 9 5 2 0 6 1 6 8 8 1 9 3 0 8 0 0
	1 1
Alimentary System	
Esophagus	+ + + + M + + + + + + + + + + + + + + + + + +
Gallbladder	A M M M A A + + + A A A A A A A A M A A + A +
Intestine large	A + A A A + A + + A + + A A A + A + A + A + A +
Intestine large, cecum	A A A A A A A + + A A + A A A + A A A A + A +
Intestine large, colon	A A A A A + A + + A + + A A A + A + A + A + A +
Leiomyosarcoma	
Intestine large, rectum	A + A A A M M I M A + M M A A M A + A A A + A +
Intestine small	A A A A A A A + + A A + A A A + A A A A + A +
Intestine small, duodenum	A A A A A A A + + A A + A A A A A A A A + A +
Intestine small, ileum	A A A A A A A + + A A + A A A A A A A A + A +
Intestine small, jejunum	A A A A A A A + + A A + A A A A A A A A + A +
Liver	+ + + A + + + + + + + + + + + A + + + + + + +
Hemangioma	
Hepatocellular carcinoma	
Hepatocellular adenoma	
Pancreas	+ + + A A + + + + + + + I M + + A + A + A + + I
Salivary glands	+ +
Stomach	+ + + A + + + + + + + + + + + A + + + + + + +
Stomach, forestomach	+ + + A + + + + + + + + + + + A + + + + + + +
Stomach, glandular	A + + A A + + + + A A + + A + + A + + + + + A +
Cardiovascular System	
Heart	+ +
Alveolar/bronchiolar carcinoma, metastatic, lung	X
Endocrine System	
Adrenal gland	+ + + A + + + + + + + + + + + A + + + + + + +
Adrenal gland, cortex	+ + + A + + + + + + + M + + + A + + + + + + +
Adrenal gland, medulla	+ + + A + + + + + + + + + + + A + + + + + + +
Islets, pancreatic	M I + A A I I + + M I M M M + + A + M I M M I I
Parathyroid gland	I + + A M M + + M M M + + M + + M M I + M I + M
Pituitary gland	M + + + + + + + + + + + I + + + M + + M I + + +
Adenoma	
Carcinoma	
Thyroid gland	+ + + A + + + + + + + + + + + + + + + + + + +
Follicular cell, adenoma	

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D-14

Talc, NTP TR 421

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Talc: 6 mg/m³ (continued)

Number of Days on Study	0 0 4 4 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 7 7 7
	2 9 2 9 0 3 4 5 5 6 1 2 2 4 4 6 7 7 8 9 9 0 0 1
	0 2 2 1 0 4 8 4 9 4 8 1 8 1 5 5 6 8 6 2 9 9 9 2
Carcass ID Number	1 0 0 1 0 1 1 1 0 1 0 1 0 0 1 1 0 1 1 0 0 1 1 0
	1 5 1 1 5 4 7 4 1 7 2 2 5 8 3 0 5 7 3 8 6 1 4 2
	7 5 6 5 0 7 6 8 9 5 2 0 6 1 6 8 8 1 9 3 0 8 0 0
	1 1
General Body System Tissue NOS	
Genital System	
Ovary	+ + + A + + + + + + + M + + + A + + + + + + +
Adenoma	
Cystadenoma	
Uterus	+ + + A + + + + + + + + + A + A + + + + + + +
Hematopoietic System	
Bone marrow	+ + + A + + A + + A + + + A + + + + + + + + +
Lymph node	M + A +
Lymph node, bronchial	M M M + + + M I + + M + + M + + + + M + + + + I
Adenocarcinoma, metastatic, kidney	X
Alveolar/bronchiolar carcinoma, metastatic, lung	X X X
Lymph node, mandibular	M + M M M + + + + + + + M + + + + + + M + + +
Lymph node, mediastinal	M M M + M M M M M M M + + + M M M + I M + + M
Adenocarcinoma, metastatic, kidney	X
Alveolar/bronchiolar carcinoma, metastatic, lung	X
Lymph node, mesenteric	M M A A A + M + + + + + A + + A M M + + + +
Spleen	A + + A + + + + + + + + + + + A + + + + + + +
Thymus	M M + + + I + + + + I + + + + + M M M + I + + +
Alveolar/bronchiolar carcinoma, metastatic, lung	X X
Integumentary System	
Mammary gland	+ + + A + + + + + + + + + + + M + + + + + + +
Skin	+ + + A + + + + + + + + + + + M + + + + + + +
Musculoskeletal System	
Bone	+ +
Vertebra, alveolar/bronchiolar carcinoma, metastatic, lung	X
Nervous System	
Brain	+ +

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D-16

Talc, NTP TR 421

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Talc: 6 mg/m³ (continued)

Number of Days on Study	0 0 4 4 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 7 7 7
	2 9 2 9 0 3 4 5 5 6 1 2 2 4 4 6 7 7 8 9 9 0 0 1
	0 2 2 1 0 4 8 4 9 4 8 1 8 1 5 5 6 8 6 2 9 9 9 2
Carcass ID Number	1 0 0 1 0 1 1 1 0 1 0 1 0 0 1 1 0 1 1 0 0 1 1 0
	1 5 1 1 5 4 7 4 1 7 2 2 5 8 3 0 5 7 3 8 6 1 4 2
	7 5 6 5 0 7 6 8 9 5 2 0 6 1 6 8 8 1 9 3 0 8 0 0
	1 1
Respiratory System	
Larynx	+ + + A A I + + + + A + + + + + + + + + + + + +
Lung	+ +
Adenocarcinoma, metastatic, kidney	
Alveolar/bronchiolar adenoma	
Alveolar/bronchiolar carcinoma	X
Hepatocellular carcinoma, metastatic, liver	
Nose	+ + + + A + + + + + + + + + + + + + + + + + +
Trachea	+ + + A A + M + + A A + A A + + M + M + + + + +
Special Senses System	
Eye	
Harderian gland	
Adenoma	
Urinary System	
Kidney	+ + + A + + + + + + + + + + + + A + + + + + +
Adenocarcinoma	
Urinary bladder	A A + A + + + + + A + + + A A + A + + + + + +
Systemic Lesions	
Multiple organs	+ +
Lymphoma malignant lymphocytic	
Lymphoma malignant mixed	

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D-18

Talc, NTP TR 421

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Talc: 18 mg/m³

Number of Days on Study	0	0	0	0	0	0	0	4	5	5	5	5	5	5	5	6	6	6	6	6	6	7	7	7
	2	2	2	2	2	2	7	0	1	4	5	5	6	8	4	4	5	6	6	8	9	0	1	1
	0	8	8	8	8	8	3	8	6	8	4	8	9	1	2	6	5	1	5	6	2	6	6	8
Carcass ID Number	2	1	2	2	2	2	3	3	2	2	3	3	3	2	3	2	3	2	2	2	2	2	2	2
	9	9	0	0	0	0	4	4	0	3	1	5	6	7	2	3	5	2	2	3	6	9	6	5
	2	6	1	3	4	6	7	9	6	2	9	6	3	0	0	8	2	2	7	8	6	6	7	9
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Alimentary System																								
Esophagus	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	M	M	A	A	M	M	+	M	A	A	A	A	+	A	A	+	A	A	A	+	+	A	+
Intestine large	A	A	A	+	+	A	A	+	+	A	+	A	+	A	+	A	+	A	A	A	+	+	A	+
Intestine large, cecum	A	A	A	+	A	A	A	+	+	A	+	A	A	A	+	A	+	A	A	A	+	+	A	+
Leiomyoma																								
Intestine large, colon	A	A	A	+	A	A	A	I	+	A	+	A	A	A	+	A	A	A	A	A	+	+	A	+
Intestine large, rectum	A	A	A	+	+	A	A	M	I	M	M	A	+	A	+	A	+	M	A	M	+	+	M	+
Intestine small	A	A	A	+	A	A	A	+	+	A	+	A	+	A	+	A	+	A	A	A	+	+	A	+
Intestine small, duodenum	A	A	A	+	A	A	A	+	+	A	M	A	A	+	A	+	A	A	A	A	+	+	A	+
Intestine small, ileum	A	A	A	A	A	A	A	+	A	A	+	A	A	+	A	+	A	A	A	A	+	+	A	+
Intestine small, jejunum	A	A	A	+	A	A	A	+	A	A	A	+	A	+	A	+	A	A	A	A	+	+	A	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																								X
Hepatocellular adenoma																X								X
Pancreas	A	+	+	+	M	A	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	A	+	+	A	I	+	+	+	+	A	+	+	+	+	+	+
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	I	I	M	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	A	+	+	I	M	A	M	+	+	M	M	+	+	I	I	M	+	+	M	I	I	+	A	+
Parathyroid gland	+	M	M	+	+	M	M	M	M	M	+	+	+	M	M	+	+	I	+	M	M	+	+	+
Pituitary gland	+	+	+	+	M	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								X
Thyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																								
General Body System																								
Tissue NOS																								+
Hemangioma																								X
Hemangiosarcoma																								

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D-20

Talc, NTP TR 421

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Talc: 18 mg/m³ (continued)

Number of Days on Study	0 0 0 0 0 0 0 4 5 5 5 5 5 5 6 6 6 6 6 6 7 7 7
	2 2 2 2 2 2 2 7 0 1 4 5 5 6 8 4 4 5 6 6 8 9 0 1 1
	0 8 8 8 8 8 8 3 8 6 8 4 8 9 1 2 6 5 1 5 6 2 6 6 8
Carcass ID Number	2 1 2 2 2 2 2 3 3 2 2 3 3 3 2 3 2 3 2 2 2 2 2 2 2
	9 9 0 0 0 0 0 4 4 0 3 1 5 6 7 2 3 5 2 2 3 6 9 6 5
	2 6 1 3 4 6 7 9 6 2 9 6 3 0 0 8 2 2 7 8 6 6 6 7 9
	1 1
Genital System	
Ovary	+ + + + + + + + + + + + + + I + M + + M + + + +
Uterus	+ + M + + + + + + + + + + + + + + + + + + +
Carcinoma adenosquamous	
Hematopoietic System	
Bone marrow	+ + + + + + + A + A A + + A A + + + + + + + +
Lymph node	+ M +
Lymph node, bronchial	M M M + M M M + + I + + + + + + + + + + + +
Lymph node, mandibular	+ M M M + + + + + + + M M + + + + + + + + + +
Lymph node, mediastinal	M M M M M M M M M + M M M M M M + + + M + M + +
Lymph node, mesenteric	M M A + M M M + + A + A + + + M + + + + M + M + +
Spleen	+ +
Hemangiosarcoma	
Thymus	M M + M + + M M + + + + + + + + + + + M + + M I
Integumentary System	
Mammary gland	+ + M + + + + + + + + I + + + + + + + + + + +
Fibrosarcoma	
Skin	+ +
Musculoskeletal System	
Bone	+ +
Nervous System	
Brain	+ +
Spinal cord	
Thoracic, ganglioneuroma	
Respiratory System	
Larynx	+ + M + + + + + + + + + + + + + + + + + + +
Lung	+ +
Alveolar/bronchiolar adenoma	
Alveolar/bronchiolar carcinoma	X
Hemangiosarcoma, metastatic, tissue NOS	X
Nose	+ +
Trachea	+ + + + + + + + + + + + + + + A + M + + + + + +

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D-22

Talc, NTP TR 421

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Talc: 18 mg/m³ (continued)

	0	0	0	0	0	0	0	4	5	5	5	5	5	5	5	6	6	6	6	6	6	6	7	7	7
Number of Days on Study	2	2	2	2	2	2	2	7	0	1	4	5	5	6	8	4	4	5	6	6	8	9	0	1	1
	0	8	8	8	8	8	8	3	8	6	8	4	8	9	1	2	6	5	1	5	6	2	6	6	8
	2	1	2	2	2	2	2	3	3	2	2	3	3	3	2	3	2	2	2	2	2	2	2	2	2
Carcass ID Number	9	9	0	0	0	0	0	4	4	0	3	1	5	6	7	2	3	5	2	2	3	6	9	6	5
	2	6	1	3	4	6	7	9	6	2	9	6	3	0	0	8	2	2	7	8	6	6	6	7	9
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Special Senses System																									
Harderian gland																									+
Adenocarcinoma																									X
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	A	A	+	+	+	+	A	+	+	+	+	A	+	+	+	A	+	A	+	+	A	+	A	A	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic																									X
Lymphoma malignant lymphocytic																									
Lymphoma malignant mixed																									X
Lymphoma malignant undifferentiated cell type																									X

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D-24

Talc, NTP TR 421

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Talc

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Liver: Hepatocellular Adenoma			
Overall rates ^a	5/46 (11%)	1/47 (2%)	4/50 (8%)
Adjusted rates ^b	16.7%	4.3%	14.0%
Terminal rates ^c	5/30 (17%)	1/23 (4%)	2/25 (8%)
First incidence (days)	729 (I)	729 (I)	581
Life table tests ^d	P=0.565	P=0.169N	P=0.602N
Logistic regression tests ^d	P=0.603N	P=0.169N	P=0.539N
Cochran-Armitage test ^d	P=0.523N		
Fisher exact test ^d		P=0.097N	P=0.447N
Liver: Hepatocellular Carcinoma			
Overall rates	7/46 (15%)	5/47 (11%)	4/50 (8%)
Adjusted rates	19.1%	18.4%	15.4%
Terminal rates	3/30 (10%)	3/23 (13%)	3/25 (12%)
First incidence (days)	426	645	718
Life table tests	P=0.308N	P=0.487N	P=0.344N
Logistic regression tests	P=0.243N	P=0.372N	P=0.255N
Cochran-Armitage test	P=0.197N		
Fisher exact test		P=0.364N	P=0.216N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rates	11/46 (24%)	6/47 (13%)	7/50 (14%)
Adjusted rates	31.1%	22.5%	25.2%
Terminal rates	7/30 (23%)	4/23 (17%)	5/25 (20%)
First incidence (days)	426	645	581
Life table tests	P=0.329N	P=0.262N	P=0.330N
Logistic regression tests	P=0.253N	P=0.147N	P=0.227N
Cochran-Armitage test	P=0.184N		
Fisher exact test		P=0.131N	P=0.163N
Lung: Alveolar/bronchiolar Adenoma			
Overall rates	3/46 (7%)	2/49 (4%)	2/50 (4%)
Adjusted rates	10.0%	6.7%	6.4%
Terminal rates	3/30 (10%)	1/23 (4%)	1/25 (4%)
First incidence (days)	729 (I)	559	548
Life table tests	P=0.505N	P=0.589N	P=0.562N
Logistic regression tests	P=0.467N	P=0.499N	P=0.515N
Cochran-Armitage test	P=0.425N		
Fisher exact test		P=0.470N	P=0.460N
Lung: Alveolar/bronchiolar Carcinoma			
Overall rates	2/46 (4%)	4/49 (8%)	1/50 (2%)
Adjusted rates	6.7%	11.6%	2.6%
Terminal rates	2/30 (7%)	0/23 (0%)	0/25 (0%)
First incidence (days)	729 (I)	491	558
Life table tests	P=0.383N	P=0.286	P=0.539N
Logistic regression tests	P=0.325N	P=0.356	P=0.500N
Cochran-Armitage test	P=0.309N		
Fisher exact test		P=0.369	P=0.468N

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Lesions in Female Mice

D-25

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rates	5/46 (11%)	6/49 (12%)	3/50 (6%)
Adjusted rates	16.7%	17.5%	8.9%
Terminal rates	5/30 (17%)	1/23 (4%)	1/25 (4%)
First incidence (days)	729 (I)	491	548
Life table tests	P=0.337N	P=0.394	P=0.428N
Logistic regression tests	P=0.269N	P=0.519	P=0.367N
Cochran-Armitage test	P=0.235N		
Fisher exact test		P=0.545	P=0.311N
Ovary: Luteoma			
Overall rates	2/38 (5%)	0/43 (0%)	0/46 (0%)
Adjusted rates	8.0%	0.0%	0.0%
Terminal rates	2/25 (8%)	0/21 (0%)	0/24 (0%)
First incidence (days)	729 (I)	- ^e	-
Life table tests	P=0.177N	P=0.277N	P=0.246N
Logistic regression tests	P=0.177N	P=0.277N	P=0.246N
Cochran-Armitage test	P=0.146N		
Fisher exact test		P=0.217N	P=0.202N
Pituitary Gland (Unspecified Site): Adenoma			
Overall rates	5/42 (12%)	4/43 (9%)	2/48 (4%)
Adjusted rates	15.1%	18.2%	7.1%
Terminal rates	2/30 (7%)	4/22 (18%)	1/25 (4%)
First incidence (days)	683	729 (I)	665
Life table tests	P=0.239N	P=0.610	P=0.290N
Logistic regression tests	P=0.189N	P=0.604N	P=0.220N
Cochran-Armitage test	P=0.133N		
Fisher exact test		P=0.485N	P=0.166N
Pituitary Gland (Unspecified Site): Carcinoma			
Overall rates	0/42 (0%)	2/43 (5%)	0/48 (0%)
Adjusted rates	0.0%	5.5%	0.0%
Terminal rates	0/30 (0%)	0/22 (0%)	0/25 (0%)
First incidence (days)	-	534	-
Life table tests	P=0.591N	P=0.237	- ^f
Logistic regression tests	P=0.515N	P=0.274	-
Cochran-Armitage test	P=0.542N		
Fisher exact test		P=0.253	-
Pituitary Gland (Unspecified Site): Adenoma or Carcinoma			
Overall rates	5/42 (12%)	6/43 (14%)	2/48 (4%)
Adjusted rates	15.1%	22.7%	7.1%
Terminal rates	2/30 (7%)	4/22 (18%)	1/25 (4%)
First incidence (days)	683	534	665
Life table tests	P=0.216N	P=0.352	P=0.290N
Logistic regression tests	P=0.150N	P=0.451	P=0.220N
Cochran-Armitage test	P=0.111N		
Fisher exact test		P=0.517	P=0.166N

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D-26

Talc, NTP TR 421

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
All Organs: Hemangioma or Hemangiosarcoma			
Overall rates	2/46 (4%)	1/49 (2%)	3/50 (6%)
Adjusted rates	6.7%	4.3%	10.1%
Terminal rates	2/30 (7%)	1/23 (4%)	2/25 (8%)
First incidence (days)	729 (T)	729 (T)	473
Life table tests	P=0.323	P=0.593N	P=0.434
Logistic regression tests	P=0.356	P=0.593N	P=0.495
Cochran-Armitage test	P=0.399		
Fisher exact test		P=0.476N	P=0.540
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, or Undifferentiated Cell Type)			
Overall rates	7/46 (15%)	7/49 (14%)	8/50 (16%)
Adjusted rates	21.3%	26.7%	27.4%
Terminal rates	5/30 (17%)	5/23 (22%)	5/25 (20%)
First incidence (days)	509	628	642
Life table tests	P=0.358	P=0.454	P=0.387
Logistic regression tests	P=0.406	P=0.607	P=0.463
Cochran-Armitage test	P=0.514		
Fisher exact test		P=0.563N	P=0.571
All Organs: Benign Tumors			
Overall rates	18/46 (39%)	9/49 (18%)	10/50 (20%)
Adjusted rates	54.5%	36.4%	33.0%
Terminal rates	15/30 (50%)	8/23 (35%)	6/25 (24%)
First incidence (days)	683	559	548
Life table tests	P=0.148N	P=0.125N	P=0.145N
Logistic regression tests	P=0.094N	P=0.044N	P=0.071N
Cochran-Armitage test	P=0.050N		
Fisher exact test		P=0.022N	P=0.033N
All Organs: Malignant Tumors			
Overall rates	19/46 (41%)	19/49 (39%)	15/50 (30%)
Adjusted rates	51.9%	55.4%	45.6%
Terminal rates	13/30 (43%)	9/23 (39%)	8/25 (32%)
First incidence (days)	426	491	473
Life table tests	P=0.372N	P=0.340	P=0.441N
Logistic regression tests	P=0.241N	P=0.546N	P=0.279N
Cochran-Armitage test	P=0.143N		
Fisher exact test		P=0.483N	P=0.173N
All Organs: Benign or Malignant Tumors			
Overall rates	31/46 (67%)	26/49 (53%)	21/50 (42%)
Adjusted rates	81.4%	75.1%	58.9%
Terminal rates	23/30 (77%)	15/23 (65%)	11/25 (44%)
First incidence (days)	426	491	473
Life table tests	P=0.141N	P=0.537	P=0.168N
Logistic regression tests	P=0.036N	P=0.162N	P=0.035N
Cochran-Armitage test	P=0.011N		
Fisher exact test		P=0.112N	P=0.011N

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Lesions in Female Mice

D-27

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Talc (continued)

- (T) Terminal sacrifice
a Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
b Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
c Observed incidence at terminal kill
d Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
e Not applicable; no tumors in animal group
f Value of statistic cannot be computed.

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D-28

Talc, NTP TR 421

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Talc^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Moribund	2	4	4
Natural deaths	17	21	21
Survivors			
Terminal sacrifice	30	23	25
Missing	1	1	
Culled			
Animals examined microscopically	46	48	50
Alimentary System			
Intestine large, cecum	(35)	(29)	(34)
Hyperplasia, lymphoid			1 (3%)
Serosa, inflammation, suppurative		1 (3%)	
Intestine large, colon	(38)	(33)	(32)
Serosa, inflammation, suppurative		2 (6%)	
Intestine small, duodenum	(27)	(25)	(27)
Ulcer, focal	1 (4%)		
Mucosa, atrophy	2 (7%)	6 (24%)	4 (15%)
Serosa, inflammation, suppurative		2 (8%)	
Intestine small, ileum	(33)	(27)	(31)
Hyperplasia, lymphoid	1 (3%)	1 (4%)	
Mucosa, atrophy	4 (12%)	6 (22%)	6 (19%)
Peyer's patch, necrosis			1 (3%)
Serosa, inflammation, suppurative		2 (7%)	1 (3%)
Intestine small, jejunum	(33)	(28)	(31)
Mucosa, atrophy	2 (6%)	7 (25%)	3 (10%)
Serosa, inflammation, suppurative		2 (7%)	1 (3%)
Liver	(46)	(46)	(50)
Eosinophilic focus		1 (2%)	
Fibrosis, focal		1 (2%)	
Focal cellular change	2 (4%)	3 (7%)	1 (2%)
Hematopoietic cell proliferation	1 (2%)	2 (4%)	2 (4%)
Inflammation, focal	1 (2%)	2 (4%)	1 (2%)
Necrosis, focal	1 (2%)	2 (4%)	2 (4%)
Pigmentation, hemosiderin, focal		1 (2%)	
Centrilobular, degeneration		1 (2%)	
Centrilobular, necrosis, coagulative		1 (2%)	
Serosa, inflammation, suppurative	4 (9%)	7 (15%)	5 (10%)
Sinusoid, inflammation	2 (4%)		
Pancreas	(42)	(39)	(44)
Inflammation, focal			2 (5%)
Acinus, hyperplasia, focal	1 (2%)		
Serosa, inflammation, suppurative	1 (2%)	5 (13%)	4 (9%)
Salivary glands	(46)	(48)	(50)
Inflammation, acute		1 (2%)	1 (2%)
Stomach	(45)	(45)	(50)
Serosa, inflammation, granulomatous			1 (2%)
Serosa, inflammation, suppurative	1 (2%)	2 (4%)	1 (2%)
Stomach, forestomach	(45)	(45)	(50)
Hyperplasia, mast cell, focal			1 (2%)
Hyperplasia, squamous, focal	2 (4%)	4 (9%)	2 (4%)
Ulcer, focal	1 (2%)	3 (7%)	

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Lesions in Female Mice

D-29

TABLE D4

Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Alimentary System (continued)			
Stomach, glandular	(45)	(39)	(46)
Inflammation, suppurative			1 (2%)
Ulcer, focal	1 (2%)	1 (3%)	
Forestomach, inflammation, focal		1 (3%)	2 (4%)
Cardiovascular System			
Heart	(46)	(48)	(50)
Myocardium, degeneration, focal	1 (2%)		
Myocardium, inflammation, focal		1 (2%)	
Myocardium, mineralization, focal	1 (2%)		
Pericardium, inflammation, suppurative	1 (2%)	2 (4%)	4 (8%)
Endocrine System			
Adrenal gland	(46)	(45)	(50)
Capsule, inflammation, suppurative	4 (9%)	7 (16%)	5 (10%)
Corticomedullary junction, hemorrhage	2 (4%)	3 (7%)	1 (2%)
Spindle cell, hyperplasia	46 (100%)	45 (100%)	47 (94%)
Adrenal gland, cortex	(46)	(44)	(50)
Cyst	2 (4%)	3 (7%)	
Inflammation, suppurative, focal			1 (2%)
Vacuolization cytoplasmic, focal	3 (7%)		
Adrenal gland, medulla	(41)	(43)	(45)
Hyperplasia, focal	2 (5%)		
Parathyroid gland	(23)	(18)	(25)
Hyperplasia	1 (4%)		
Pituitary gland	(42)	(42)	(48)
Cyst	2 (5%)		
Hemorrhage, focal	2 (5%)		
Hyperplasia, focal	2 (5%)		
Pigmentation, lipofuscin	1 (2%)		
Thyroid gland	(43)	(47)	(49)
Cyst	2 (5%)		
Inflammation, acute, focal			2 (4%)
C-cell, hyperplasia	1 (2%)		1 (2%)
Follicular cell, hyperplasia	9 (21%)	12 (26%)	10 (20%)
General Body System			
Tissue NOS	(4)	(1)	(2)
Thrombosis, chronic	1 (25%)		
Genital System			
Ovary	(38)	(43)	(46)
Abscess	4 (11%)	10 (23%)	7 (15%)
Cyst	6 (16%)	11 (26%)	10 (22%)
Thrombosis	1 (3%)	2 (5%)	
Uterus	(44)	(45)	(49)
Angiectasis			1 (2%)
Hyperplasia, histiocytic, focal			1 (2%)
Metaplasia, squamous		1 (2%)	
Thrombosis	1 (2%)		
Endometrium, hyperplasia, cystic	34 (77%)	30 (67%)	35 (71%)
Mucosa, inflammation, suppurative	3 (7%)	7 (16%)	4 (8%)
Serosa, inflammation, suppurative	1 (2%)	4 (9%)	2 (4%)

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TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Hematopoietic System			
Bone marrow	(41)	(43)	(45)
Hyperplasia	1 (2%)	4 (9%)	5 (11%)
Myelofibrosis	28 (68%)	23 (53%)	27 (60%)
Myeloid cell, hyperplasia	1 (2%)	6 (14%)	3 (7%)
Lymph node	(46)	(46)	(49)
Iliac, hyperplasia, lymphoid			1 (2%)
Iliac, inflammation	1 (2%)		1 (2%)
Pancreatic, hyperplasia, lymphoid	1 (2%)		1 (2%)
Pancreatic, infiltration cellular, mixed cell			1 (2%)
Pancreatic, follicular, necrosis			1 (2%)
Renal, hyperplasia, lymphoid		2 (4%)	2 (4%)
Renal, infiltration cellular, mixed cell			1 (2%)
Renal, inflammation	1 (2%)	1 (2%)	1 (2%)
Renal, follicular, necrosis		2 (4%)	1 (2%)
Lymph node, bronchial	(38)	(37)	(43)
Hyperplasia, histiocytic		25 (68%)	39 (91%)
Hyperplasia, lymphoid		16 (43%)	20 (47%)
Infiltration cellular, mixed cell	1 (3%)		
Inflammation, acute	1 (3%)	1 (3%)	1 (2%)
Lymph node, mandibular	(35)	(38)	(36)
Cyst			1 (3%)
Depletion lymphoid	1 (3%)		
Hyperplasia, histiocytic	1 (3%)		
Hyperplasia, lymphoid		1 (3%)	3 (8%)
Hyperplasia, plasma cell	1 (3%)		
Infiltration cellular, mixed cell		1 (3%)	
Inflammation		1 (3%)	1 (3%)
Follicular, necrosis		1 (3%)	
Lymph node, mediastinal	(13)	(17)	(14)
Hyperplasia, histiocytic	1 (8%)	3 (18%)	2 (14%)
Hyperplasia, lymphoid		1 (6%)	2 (14%)
Infiltration cellular, mixed cell	1 (8%)		
Lymph node, mesenteric	(35)	(31)	(37)
Depletion lymphoid		1 (3%)	2 (5%)
Hematocyst			1 (3%)
Hyperplasia, histiocytic		1 (3%)	1 (3%)
Hyperplasia, lymphoid		2 (6%)	2 (5%)
Hyperplasia, plasma cell			1 (3%)
Infiltration cellular, mixed cell	5 (14%)	5 (16%)	5 (14%)
Inflammation		2 (6%)	1 (3%)
Follicular, necrosis	3 (9%)	12 (39%)	7 (19%)
Spleen	(45)	(44)	(50)
Congestion	2 (4%)		
Hematopoietic cell proliferation	8 (18%)	12 (27%)	10 (20%)
Hyperplasia, lymphoid	5 (11%)	8 (18%)	6 (12%)
Inflammation, suppurative	2 (4%)		1 (2%)
Capsule, inflammation, suppurative	2 (4%)	3 (7%)	3 (6%)
Lymphoid follicle, depletion lymphoid	2 (4%)	3 (7%)	5 (10%)
Lymphoid follicle, necrosis	2 (4%)	4 (9%)	2 (4%)
Thymus	(40)	(40)	(41)
Cyst	2 (5%)	2 (5%)	
Hyperplasia, plasma cell		1 (3%)	
Inflammation, suppurative		1 (3%)	1 (2%)
Necrosis	3 (8%)	5 (13%)	
Cortex, depletion lymphoid	8 (20%)	12 (30%)	15 (37%)

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Lesions in Female Mice

D-31

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Integumentary System			
Mammary gland	(41)	(45)	(48)
Abscess			1 (2%)
Edema	1 (2%)		
Skin	(46)	(46)	(50)
Alopecia	2 (4%)	2 (4%)	
Musculoskeletal System			
Bone	(46)	(48)	(50)
Periosteum, femur, proliferation connective tissue	1 (2%)		
Nervous System			
Brain	(46)	(48)	(50)
Hydrocephalus		2 (4%)	
Mineralization, focal	36 (78%)	33 (69%)	29 (58%)
Respiratory System			
Larynx	(42)	(43)	(48)
Inflammation, acute	1 (2%)		
Lung	(46)	(48)	(50)
Congestion	1 (2%)	3 (6%)	
Hyperplasia, histiocytic			1 (2%)
Hyperplasia, macrophage	2 (4%)	45 (94%)	43 (86%)
Inflammation, chronic active		25 (52%)	38 (76%)
Metaplasia, osseous, focal	1 (2%)		
Alveolar epithelium, hyperplasia, focal			1 (2%)
Perivascular, inflammation, suppurative		3 (6%)	1 (2%)
Pleura, inflammation, suppurative	1 (2%)	2 (4%)	5 (10%)
Nose	(46)	(46)	(50)
Cytoplasmic alteration, focal	29 (63%)	37 (80%)	40 (80%)
Developmental malformation	1 (2%)		
Erosion, focal	3 (7%)		1 (2%)
Inflammation, acute	6 (13%)	4 (9%)	5 (10%)
Ulcer, focal	1 (2%)		
Special Senses System			
Eye		(1)	
Inflammation, suppurative		1 (100%)	
Harderian gland	(2)	(2)	(1)
Inflammation, suppurative		1 (50%)	
Urinary System			
Kidney	(46)	(46)	(50)
Casts protein		2 (4%)	
Infarct	1 (2%)	1 (2%)	
Inflammation, focal	1 (2%)	1 (2%)	1 (2%)
Metaplasia, osseous, focal	1 (2%)		2 (4%)
Nephropathy, chronic	1 (2%)	1 (2%)	
Capsule, inflammation, suppurative	3 (7%)	6 (13%)	5 (10%)
Renal tubule, hyperplasia, focal		1 (2%)	

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TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Talc (continued)

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Urinary System (continued)			
Urinary bladder	(44)	(40)	(41)
Serosa, inflammation, suppurative		3 (8%)	3 (7%)
Submucosa, hyperplasia, lymphoid	1 (2%)		
Submucosa, inflammation, suppurative			1 (2%)

^a Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

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E-1

APPENDIX E ORGAN WEIGHTS AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE E1	Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 6-Month Interim Evaluation in the Lifetime Inhalation Study of Talc	E-2
TABLE E2	Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 11-Month Interim Evaluation in the Lifetime Inhalation Study of Talc	E-3
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E-2

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TABLE E1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 6-Month Interim Evaluation in the Lifetime Inhalation Study of Talc^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
n	3	3	3
Necropsy body wt	379 ± 2	365 ± 9	351 ± 4*
Brain			
Absolute	2.061 ± 0.073	1.962 ± 0.035	1.964 ± 0.041
Relative	5.44 ± 0.22	5.38 ± 0.22	5.59 ± 0.10
Heart			
Absolute	1.087 ± 0.024	0.984 ± 0.047	1.008 ± 0.018
Relative	2.87 ± 0.07	2.69 ± 0.07	2.87 ± 0.03
R. Kidney			
Absolute	1.203 ± 0.055	1.155 ± 0.028	1.143 ± 0.025
Relative	3.17 ± 0.16	3.16 ± 0.01	3.25 ± 0.04
Liver			
Absolute	12.969 ± 0.336	11.658 ± 0.483	11.644 ± 0.613
Relative	34.20 ± 0.79	31.89 ± 0.65	33.11 ± 1.43
Lungs			
Absolute	1.196 ± 0.049	1.201 ± 0.060	1.600 ± 0.073**
Relative	3.15 ± 0.11	3.29 ± 0.19	4.55 ± 0.19**
Female			
n	3	3	3
Necropsy body wt	216 ± 10	210 ± 5	212 ± 7
Brain			
Absolute	1.801 ± 0.020	1.800 ± 0.030	1.860 ± 0.031
Relative	8.39 ± 0.33	8.57 ± 0.28	8.82 ± 0.39
Heart			
Absolute	0.679 ± 0.023	0.691 ± 0.031	0.716 ± 0.055
Relative	3.16 ± 0.11	3.29 ± 0.13	3.38 ± 0.20
R. Kidney			
Absolute	0.700 ± 0.043	0.775 ± 0.025	0.751 ± 0.030
Relative	3.25 ± 0.17	3.69 ± 0.10	3.55 ± 0.07
Liver			
Absolute	7.579 ± 0.502	7.253 ± 0.172	6.875 ± 0.409
Relative	35.13 ± 1.09	34.51 ± 0.33	32.47 ± 1.21
Lungs			
Absolute	1.006 ± 0.112	0.986 ± 0.064	1.090 ± 0.010
Relative	4.71 ± 0.65	4.69 ± 0.29	5.17 ± 0.21

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test

** P≤0.01

^a Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

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Organ Weight Analyses

E-3

TABLE E2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 11-Month Interim Evaluation in the Lifetime Inhalation Study of Tale^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
n	2	3	3
Necropsy body wt	425 ± 10	406 ± 15	395 ± 14
Brain			
Absolute	2.018 ± 0.010	1.616 ± 0.306	2.020 ± 0.012
Relative	4.75 ± 0.13	3.97 ± 0.74	5.13 ± 0.16
Heart			
Absolute	1.161 ± 0.080	1.051 ± 0.063	1.079 ± 0.048
Relative	2.73 ± 0.12	2.58 ± 0.06	2.73 ± 0.09
R. Kidney			
Absolute	1.313 ± 0.008	1.242 ± 0.062	1.216 ± 0.069
Relative	3.09 ± 0.09	3.07 ± 0.26	3.07 ± 0.07
Liver			
Absolute	12.824 ± 0.065	12.454 ± 0.424	12.223 ± 0.618
Relative	30.20 ± 0.86	30.72 ± 1.47	30.92 ± 0.50
Lungs			
Absolute	1.228 ± 0.143	1.152 ± 0.043	1.979 ± 0.077**
Relative	2.90 ± 0.40	2.85 ± 0.18	5.02 ± 0.16**
Female			
n	3	3	3
Necropsy body wt	254 ± 7	249 ± 5	247 ± 10
Brain			
Absolute	1.863 ± 0.003	1.867 ± 0.036	1.845 ± 0.030
Relative	7.36 ± 0.22	7.52 ± 0.18	7.50 ± 0.19
Heart			
Absolute	0.858 ± 0.032	0.796 ± 0.020	0.753 ± 0.063
Relative	3.38 ± 0.06	3.20 ± 0.06	3.05 ± 0.19
R. Kidney			
Absolute	0.830 ± 0.007	0.839 ± 0.002	0.735 ± 0.034*
Relative	3.28 ± 0.11	3.38 ± 0.07	2.99 ± 0.13
Liver			
Absolute	7.878 ± 0.275	7.774 ± 0.130	7.537 ± 0.354
Relative	31.13 ± 1.53	31.30 ± 0.47	30.57 ± 0.50
Lungs			
Absolute	0.959 ± 0.037	1.039 ± 0.034	1.551 ± 0.163**
Relative	3.79 ± 0.20	4.18 ± 0.09	6.27 ± 0.48**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test

** $P \leq 0.01$

^a Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

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E-4

Talc, NTP TR 421

TABLE E3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 18-Month Interim Evaluation in the Lifetime Inhalation Study of Talc^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
n	3	3	2
Necropsy body wt	446 ± 14	428 ± 10	430 ± 2
Brain			
Absolute	2.019 ± 0.043	1.965 ± 0.035	2.092 ± 0.004
Relative	4.53 ± 0.10	4.60 ± 0.17	4.86 ± 0.01
Heart			
Absolute	1.077 ± 0.065	1.027 ± 0.030	1.131 ± 0.103
Relative	2.41 ± 0.09	2.40 ± 0.07	2.63 ± 0.23
R. Kidney			
Absolute	1.913 ± 0.599	1.328 ± 0.063	1.317 ± 0.023
Relative	4.27 ± 1.31	3.10 ± 0.12	3.06 ± 0.06
Liver			
Absolute	14.329 ± 1.434	13.866 ± 0.882	12.520 ± 0.189
Relative	32.10 ± 3.01	32.38 ± 1.68	29.10 ± 0.56
Lungs			
Absolute	1.691 ± 0.100	1.852 ± 0.058	3.169 ± 0.121**
Relative	3.78 ± 0.13	4.34 ± 0.21	7.36 ± 0.25**
Female			
n	3	3	3
Necropsy body wt	305 ± 5	275 ± 4**	280 ± 4*
Brain			
Absolute	1.840 ± 0.028	1.827 ± 0.045	1.847 ± 0.013
Relative	6.04 ± 0.17	6.63 ± 0.11*	6.61 ± 0.13*
Heart			
Absolute	0.772 ± 0.015	0.706 ± 0.010*	0.765 ± 0.011
Relative	2.53 ± 0.08	2.56 ± 0.03	2.74 ± 0.01*
R. Kidney			
Absolute	0.929 ± 0.023	0.902 ± 0.038	0.955 ± 0.047
Relative	3.05 ± 0.12	3.28 ± 0.17	3.41 ± 0.13
Liver			
Absolute	8.750 ± 0.223	8.540 ± 0.648	8.904 ± 0.596
Relative	28.71 ± 0.35	31.03 ± 2.47	31.84 ± 1.94
Lungs			
Absolute	1.130 ± 0.026	1.395 ± 0.046**	2.600 ± 0.030**
Relative	3.71 ± 0.12	5.07 ± 0.11**	9.31 ± 0.18**

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test

** P≤0.01

^a Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

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Organ Weight Analyses

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TABLE E4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 24-Month Interim Evaluation in the Lifetime Inhalation Study of Talc^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
n	3	6	2
Necropsy body wt	406 ± 29	422 ± 12	392 ± 30
Brain			
Absolute	2.068 ± 0.015	2.023 ± 0.025	1.989 ± 0.008
Relative	5.15 ± 0.42	4.81 ± 0.11	5.10 ± 0.37
Heart			
Absolute	1.065 ± 0.022	1.126 ± 0.044	0.993 ± 0.026
Relative	2.66 ± 0.25	2.69 ± 0.18	2.54 ± 0.13
R. Kidney			
Absolute	1.720 ± 0.138	1.577 ± 0.048	1.649 ± 0.068
Relative	4.25 ± 0.32	3.76 ± 0.19	4.24 ± 0.50
Liver			
Absolute	15.298 ± 0.187	14.924 ± 0.480	14.344 ± 1.253
Relative	38.11 ± 3.23	35.55 ± 1.80	37.05 ± 6.03
Lungs			
Absolute	1.766 ± 0.177	2.150 ± 0.230	2.473 ± 0.674
Relative	4.40 ± 0.55	5.18 ± 0.69	6.48 ± 2.21
Female			
n	5	9	3
Necropsy body wt	296 ± 17	296 ± 10	262 ± 25
Brain			
Absolute	1.821 ± 0.023	1.826 ± 0.011	1.865 ± 0.012
Relative	6.24 ± 0.42	6.24 ± 0.21	7.23 ± 0.63
Heart			
Absolute	0.826 ± 0.014	0.826 ± 0.032	0.824 ± 0.045
Relative	2.83 ± 0.19	2.81 ± 0.10	3.16 ± 0.13
R. Kidney			
Absolute	1.118 ± 0.055	1.137 ± 0.044	1.021 ± 0.022
Relative	3.82 ± 0.26	3.85 ± 0.10	3.97 ± 0.44
Liver			
Absolute	11.218 ± 0.527	12.127 ± 0.672	9.966 ± 0.246
Relative	38.38 ± 2.74	41.16 ± 2.12	38.84 ± 4.59
Lungs			
Absolute	1.014 ± 0.104	1.447 ± 0.219	3.261 ± 0.115**
Relative	3.40 ± 0.23	4.88 ± 0.67	12.73 ± 1.62**

** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test

^a Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

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TABLE E5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the Termination
of the Lifetime Inhalation Study of Talc^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
n	8	12	13
Necropsy body wt	379 ± 17	397 ± 6	326 ± 12**
Brain			
Absolute	2.030 ± 0.016	2.041 ± 0.015	2.014 ± 0.019
Relative	5.45 ± 0.28	5.16 ± 0.09	6.29 ± 0.25*
Heart			
Absolute	1.385 ± 0.104	1.288 ± 0.041	1.302 ± 0.064
Relative	3.68 ± 0.26	3.26 ± 0.13	4.05 ± 0.22
R. Kidney			
Absolute	1.899 ± 0.151	1.847 ± 0.125	1.737 ± 0.101
Relative	5.09 ± 0.49	4.69 ± 0.37	5.39 ± 0.35
Liver			
Absolute	15.501 ± 0.861	16.562 ± 0.540	14.055 ± 0.936
Relative	41.03 ± 1.67	41.92 ± 1.73	42.85 ± 1.76
Lungs			
Absolute	2.154 ± 0.124	2.509 ± 0.068	4.026 ± 0.196**
Relative	5.76 ± 0.38	6.34 ± 0.21	12.65 ± 0.85**
Female			
n	12	13	9
Necropsy body wt	260 ± 14	247 ± 14	231 ± 9
Brain			
Absolute	1.975 ± 0.122	1.860 ± 0.020	1.847 ± 0.028
Relative	8.03 ± 0.95	7.89 ± 0.51	8.06 ± 0.27
Heart			
Absolute	1.020 ± 0.039	1.006 ± 0.026	1.047 ± 0.027
Relative	4.03 ± 0.24	4.33 ± 0.39	4.58 ± 0.20
R. Kidney			
Absolute	1.313 ± 0.047	1.235 ± 0.049	1.281 ± 0.079
Relative	5.21 ± 0.34	5.22 ± 0.36	5.66 ± 0.55
Liver			
Absolute	12.005 ± 0.660	12.567 ± 0.903	12.313 ± 0.642
Relative	46.35 ± 1.68	51.25 ± 2.90	53.69 ± 3.72
Lungs			
Absolute	1.575 ± 0.109	2.673 ± 0.362**	4.050 ± 0.228**
Relative	6.11 ± 0.35	11.77 ± 2.10*	17.83 ± 1.43**

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test

** P≤0.01

^a Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

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Organ Weight Analyses

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TABLE E6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 6-Month Interim Evaluation in the 2-Year Inhalation Study of Talc^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
n	4	4	4
Necropsy body wt	31.3 ± 0.9	31.1 ± 0.9	32.1 ± 0.6
Brain			
Absolute	0.431 ± 0.028	0.458 ± 0.006	0.469 ± 0.008
Relative	13.81 ± 0.90	14.74 ± 0.23	14.60 ± 0.38
Heart			
Absolute	0.159 ± 0.003	0.165 ± 0.008	0.157 ± 0.011
Relative	5.10 ± 0.07	5.31 ± 0.33	4.88 ± 0.25
R. Kidney			
Absolute	0.303 ± 0.022	0.297 ± 0.018	0.292 ± 0.011
Relative	9.66 ± 0.40	9.58 ± 0.70	9.10 ± 0.33
Liver			
Absolute	1.737 ± 0.079	1.792 ± 0.066	1.731 ± 0.060
Relative	55.51 ± 1.06	57.75 ± 2.77	53.84 ± 1.19
Lungs			
Absolute	0.165 ± 0.008	0.149 ± 0.010	0.173 ± 0.017
Relative	5.29 ± 0.35	4.78 ± 0.27	5.35 ± 0.44
Female			
n	4	4	4
Necropsy body wt	27.1 ± 0.9	27.2 ± 1.7	29.5 ± 1.4
Brain			
Absolute	0.474 ± 0.007	0.482 ± 0.008	0.474 ± 0.019
Relative	17.52 ± 0.36	17.85 ± 0.81	16.10 ± 0.67
Heart			
Absolute	0.142 ± 0.004	0.133 ± 0.005	0.145 ± 0.006
Relative	5.27 ± 0.30	4.92 ± 0.19	4.92 ± 0.15
R. Kidney			
Absolute	0.201 ± 0.011	0.203 ± 0.004	0.217 ± 0.008
Relative	7.40 ± 0.20	7.53 ± 0.34	7.37 ± 0.13
Liver			
Absolute	1.541 ± 0.099	1.640 ± 0.138	1.628 ± 0.033
Relative	56.86 ± 2.92	60.01 ± 1.74	55.38 ± 1.91
Lungs			
Absolute	0.190 ± 0.019	0.164 ± 0.011	0.178 ± 0.011
Relative	7.11 ± 0.96	6.03 ± 0.28	6.04 ± 0.26

^a Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

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TABLE E7
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 12-Month Interim Evaluation
in the 2-Year Inhalation Study of Talc^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
n	4	4	4
Necropsy body wt	34.6 ± 1.7	37.2 ± 0.3	33.1 ± 1.3
Brain			
Absolute	0.478 ± 0.020	0.475 ± 0.009	0.475 ± 0.009
Relative	13.87 ± 0.31	12.76 ± 0.16	14.39 ± 0.38
Heart			
Absolute	0.196 ± 0.023	0.195 ± 0.005	0.205 ± 0.023
Relative	5.62 ± 0.37	5.23 ± 0.10	6.21 ± 0.69
R. Kidney			
Absolute	0.334 ± 0.007	0.339 ± 0.020	0.311 ± 0.027
Relative	9.71 ± 0.28	9.12 ± 0.52	9.41 ± 0.86
Liver			
Absolute	1.612 ± 0.052	1.886 ± 0.124	1.928 ± 0.240
Relative	46.77 ± 0.79	50.72 ± 3.25	58.55 ± 8.01
Lungs			
Absolute	0.157 ± 0.009	0.216 ± 0.018	0.243 ± 0.032*
Relative	4.54 ± 0.17	5.80 ± 0.46	7.30 ± 0.72**
Female			
n	3	4	4
Necropsy body wt	32.1 ± 2.4	33.3 ± 1.3	28.7 ± 1.2
Brain			
Absolute	0.478 ± 0.006	0.488 ± 0.005	0.491 ± 0.008
Relative	15.04 ± 1.16	14.74 ± 0.70	17.16 ± 0.55
Heart			
Absolute	0.151 ± 0.004	0.162 ± 0.008	0.190 ± 0.010*
Relative	4.72 ± 0.23	4.91 ± 0.42	6.64 ± 0.47*
R. Kidney			
Absolute	0.225 ± 0.010	0.231 ± 0.008	0.230 ± 0.011
Relative	7.03 ± 0.22	6.97 ± 0.40	8.01 ± 0.10
Liver			
Absolute	1.470 ± 0.105	1.796 ± 0.036*	1.477 ± 0.093
Relative	46.04 ± 3.71	54.20 ± 2.55	51.40 ± 2.48
Lungs			
Absolute	0.151 ± 0.019	0.191 ± 0.014	0.207 ± 0.015*
Relative	4.68 ± 0.23	5.78 ± 0.61	7.19 ± 0.24**

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test

** P≤0.01

^a Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

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Organ Weight Analyses

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TABLE E8
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 18-Month Interim Evaluation in the 2-Year Inhalation Study of Talc^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
n	4	4	4
Necropsy body wt	33.1 ± 3.0	37.5 ± 2.1	35.4 ± 1.7
Brain			
Absolute	0.467 ± 0.007	0.470 ± 0.009	0.496 ± 0.014
Relative	14.51 ± 1.44	12.63 ± 0.58	14.10 ± 0.76
Heart			
Absolute	0.193 ± 0.017	0.186 ± 0.011	0.203 ± 0.006
Relative	6.18 ± 1.29	5.00 ± 0.35	5.77 ± 0.22
R. Kidney			
Absolute	0.342 ± 0.007	0.361 ± 0.021	0.350 ± 0.009
Relative	10.66 ± 1.23	9.66 ± 0.47	9.91 ± 0.22
Liver			
Absolute	1.844 ± 0.228	1.796 ± 0.080	1.748 ± 0.113
Relative	57.08 ± 7.95	48.07 ± 1.26	49.28 ± 1.45
Lungs			
Absolute	0.229 ± 0.034	0.238 ± 0.013	0.345 ± 0.032*
Relative	7.45 ± 2.01	6.42 ± 0.57	9.79 ± 0.91
Female			
n	4	4	4
Necropsy body wt	32.1 ± 1.2	31.9 ± 1.6	27.6 ± 1.0*
Brain			
Absolute	0.483 ± 0.007	0.467 ± 0.019	0.501 ± 0.038
Relative	15.10 ± 0.59	14.73 ± 0.90	18.33 ± 1.91
Heart			
Absolute	0.155 ± 0.008	0.154 ± 0.011	0.164 ± 0.010
Relative	4.85 ± 0.28	4.87 ± 0.47	5.96 ± 0.48
R. Kidney			
Absolute	0.238 ± 0.009	0.233 ± 0.011	0.228 ± 0.007
Relative	7.41 ± 0.28	7.35 ± 0.45	8.32 ± 0.55
Liver			
Absolute	1.446 ± 0.056	1.592 ± 0.034	1.318 ± 0.055 ^b
Relative	45.10 ± 1.35	50.17 ± 2.02	48.69 ± 0.30 ^b
Lungs			
Absolute	0.223 ± 0.008	0.242 ± 0.018	0.299 ± 0.018**
Relative	6.96 ± 0.07	7.65 ± 0.73	10.90 ± 0.87**

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test

** P≤0.01

^a Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)^b n=3

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TABLE E9
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the Termination
of 2-Year Inhalation Study of Talc^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
n	30	28	32
Necropsy body wt	33.4 ± 0.5	32.1 ± 0.8	31.2 ± 0.4**
Brain			
Absolute	0.461 ± 0.004	0.458 ± 0.004	0.460 ± 0.005
Relative	13.90 ± 0.22	14.50 ± 0.34	14.78 ± 0.19*
Heart			
Absolute	0.183 ± 0.003	0.181 ± 0.004	0.183 ± 0.005
Relative	5.52 ± 0.12	5.68 ± 0.10	5.88 ± 0.15
R. Kidney			
Absolute	0.361 ± 0.010	0.362 ± 0.010	0.354 ± 0.006
Relative	10.85 ± 0.27	11.28 ± 0.16	11.34 ± 0.18
Liver			
Absolute	1.845 ± 0.064	1.733 ± 0.073 ^b	1.535 ± 0.033 ^{*,c}
Relative	55.64 ± 2.21	53.14 ± 1.72 ^b	49.27 ± 1.03 ^c
Lungs			
Absolute	0.252 ± 0.008 ^c	0.258 ± 0.009 ^b	0.408 ± 0.011 ^{**}
Relative	7.47 ± 0.25 ^c	8.01 ± 0.24 ^b	13.08 ± 0.33 ^{**}
Female			
n	30	23	25
Necropsy body wt	31.4 ± 0.6	31.7 ± 0.7	30.7 ± 0.5
Brain			
Absolute	0.484 ± 0.004	0.469 ± 0.006	0.477 ± 0.003
Relative	15.53 ± 0.26	14.93 ± 0.28	15.59 ± 0.20
Heart			
Absolute	0.164 ± 0.005	0.190 ± 0.009 ^{**}	0.163 ± 0.003
Relative	5.24 ± 0.15	6.02 ± 0.28 ^{**}	5.32 ± 0.09
R. Kidney			
Absolute	0.251 ± 0.007 ^d	0.265 ± 0.010	0.257 ± 0.007 ^e
Relative	8.03 ± 0.17 ^d	8.38 ± 0.27	8.37 ± 0.14 ^e
Liver			
Absolute	1.816 ± 0.089	1.770 ± 0.107 ^f	1.761 ± 0.083 ^e
Relative	57.41 ± 2.25	55.45 ± 3.13 ^f	56.94 ± 1.93 ^e
Lungs			
Absolute	0.276 ± 0.014	0.293 ± 0.012	0.410 ± 0.010 ^{**}
Relative	8.80 ± 0.42	9.28 ± 0.34	13.39 ± 0.28 ^{**}

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test

** P≤0.01

^a Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)^b n=27^c n=28^d n=29^e n=24^f n=22

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APPENDIX F

LUNG BURDEN, PULMONARY FUNCTION, AND LUNG BIOCHEMISTRY IN RATS

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METHODS

Lung Burden

Lung talc burden was measured to determine the relationship between the exposure concentration and the amount of talc deposited and retained within the pulmonary region of the respiratory tract. The method used for analyzing for talc in lungs has been published (Hanson *et al.*, 1985). Lung burdens were determined on 3 male and 3 female rats from each exposure group sacrificed at 27, 47, 79, and 105 weeks after the start of exposure. The analysis was based on determination of acid insoluble magnesium in the lung. MRI reported that the value for the magnesium was 19.33% for batch 02 and 19.47% for batch 03. These values and the results of the analysis at LITRI were close to the theoretical value of magnesium for talc (19.22%). Since rats sacrificed at 27, 47, and 79 weeks had been exposed to only batch 02 of talc, 19.33% magnesium was used to calculate the quantity of talc for these rats. Because batch 03 was used for the last 4 months of exposure and lung burdens of rats after 105 weeks of exposure to talc would be expected to contain substantial amounts of batch 03 talc, 19.47% magnesium was used to calculate the quantity of talc deposited in the lungs of these rats.

All operations in conjunction with tissue analysis for talc were done while wearing talc-free gloves. Left lung lobes were weighed at necropsy and stored frozen (-20° C) until used. Lungs were homogenized using water and the proteins were precipitated with 70% perchloric acid. The individual samples were filtered and washed with 5% trichloroacetic acid (TCA) to remove perchlorates. Washing continued until magnesium levels in the wash were within 10% of levels in the TCA solution (≤ 0.03 ppm magnesium). Filters and tissue residues were placed in 15 mL porcelain crucibles, dried slowly (200° C), and then ashed at 600° C for 1 hour. Ashed samples were transferred to Teflon beakers using 2 mL HCl and evaporated to dryness. Samples were then digested in hydrofluoric acid (HF), and the HF evaporated. Additional HF was added and reevaporated. Sulfuric acid was added to remove trace HF, and samples were then diluted with distilled water and analyzed for magnesium by atomic absorbance (Perkin Elmer, Model 306, Atomic Absorption Spectrophotometer) with a magnesium hollow cathode lamp and an air acetylene flame (Hanson *et al.*, 1985).

Pulmonary Function

Ten male and 10 female rats from each exposure level were assigned for respiratory function analyses. Respiratory function was measured at 6 months (27 weeks), 10 months (43 weeks), and 18 months (79 weeks). At 24 months (103 to 104 weeks) of exposure, respiratory function was performed on all surviving rats not assigned to the lifetime study. Respiratory function was measured by noninvasive techniques, using methods previously published (Harkema *et al.*, 1982).

Tests were conducted using a 1.4 L combination flow and pressure plethysmograph. Flows were measured by measuring differential pressures across a wire screen pneumotachograph in the plethysmograph wall. Volumes were obtained by integration (Model 6, Pulmonary Mechanics Analyzer, Buxco Electronics, Sharon, CT). In the pressure mode, used only for measuring functional residual capacity, the pneumotachograph hole was sealed and volume changes were measured as pressure changes. The plethysmograph was maintained at approximately 37° C by a resistance element. Transpulmonary pressure was measured using transducers connected to the external airway and a liquid-filled, 2.2 mm O.D. esophageal catheter.

A positive-negative pressure respirator system was used to induce quasistatic and forced respiratory movements, simulating the movements performed voluntarily by man. Reservoirs maintained at +40 and -50 cm H₂O were connected to the airway by solenoid valves. Inspiratory and quasistatic expiratory flow rates were limited by calibrated needle valves to 5 and 3 mL/sec, respectively. Inspirations were stopped automatically at a transpulmonary pressure of 30 cm H₂O, defining the lung volume at that distending pressure as total lung capacity (TLC). Forced inhalations were induced from TLC by opening the airway to the negative pressure reservoir via a rapidly opening valve having a 9.5 mm I.D., with no intentional flow restriction between the valve and the reservoir.

The rats were anesthetized with halothane and intubated orally with a tracheal catheter 5.5 cm long \times 1.8 mm I.D., fabricated from a 14-gauge intravenous catheter as previously described (Mauderly, 1977).

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Lung Burden, Pulmonary Function, and Lung Biochemistry in Rats

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The breathing port in the plethysmograph wall was a luer fitting drilled to 2.5 mm I.D. The frequency response of the plethysmograph-respirator-tracheal catheter system has been tested and found adequate for forced expiratory events in rats. No phase lag among flow, pressure and volume signals has been found in the frequency range of spontaneous breathing.

Rats were anesthetized, intubated and placed prone in the plethysmograph. The esophageal catheter was adjusted to maximize the recorded transpulmonary pressure signal. Anesthetic depth was adjusted to yield a respiratory frequency of 50 to 60 per minute. Respiratory frequency, tidal volume, minute volume, dynamic lung compliance, and total pulmonary resistance were recorded during spontaneous respiration, time-averaged by a data logger and displayed on a teletype terminal.

Prior to each subsequent measurement procedure, the rat's lung was manually inflated with a syringe to induce apnea. A quasistatic deflation from TLC to residual volume allowed measurement of vital capacity and the quasistatic expiratory pressure-volume curve. Quasistatic lung chord compliance was measured as the slope of the curve over the chord between the apneic lung volume and the volume at +10 cm H₂O pressure. Maximum quasistatic compliance was measured as the steepest slope of the pressure-volume curve over any 2 cm H₂O pressure interval. Functional residual capacity was measured by the barometric method (Dubois *et al.*, 1956) from recordings of lung volume and airway pressure changes as the rat resumed breathing against a blocked airway. From these measurements, all subdivisions of lung volume were calculated including residual volume.

Alveolar-capillary gas exchange was evaluated by a single-breath, CO diffusing capacity test (Ogilvie *et al.*, 1957). The lungs were inflated with a gas mixture containing CO and Ne in air to 20 cm H₂O transpulmonary pressure. After 6 seconds, one-half of the gas was withdrawn and the remaining gas collected for analysis by gas chromatography. The lung volume when inflated with the mixture was measured by neon dilution.

A forced inhalation was performed as described above, and the maneuver analyzed by a microprocessor in the data logger (Model D-12, Buxco). Data included forced vital capacity (FVC), the percentage of FVC exhaled in 0.1 second, flow rates at peak flow, and at 50%, 25%, and 10% of FVC.

A single-breath nitrogen washout was performed by recording volume and nitrogen concentration of expirate during a slow deflation after an inflation to TLC with oxygen. The slope of phase III ("alveolar plateau") of the washout curve was calculated to assess the uniformity of intrapulmonary gas distribution.

Lung Biochemistry

All surviving rats from each exposure group (the 3 males and 3 females originally assigned for lung burden/histology and the 10 males and 10 females from physiology/biochemistry) were sacrificed after 105 weeks of exposure.

The rats were anesthetized with halothane and sacrificed by exsanguination from the abdominal aorta or renal artery. The heart and lung block was removed, the right apical, right cardiac, and right intermediate portions of each rat lung were given endobronchial saline lavage (6 mL total volume in three, 2.0 mL washes of saline), and the bronchoalveolar lavage (BAL) fluid was centrifuged at 300 × G to separate the cells from the supernatant fluid.

Airway Fluid Enzymes and Cytology Measurements

In this study, BAL fluid was analyzed to determine the degree of:

- 1) Cell injury as indicated by concentration of lactate dehydrogenase (LDHP)
- 2) Chronic inflammatory response as indicated by presence of increased numbers of polymorphonuclear leukocytes (PMN) and pulmonary alveolar macrophages (AM) as well as increased protein and alkaline phosphatase activity

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- 3) Lysosomal activation as indicated by β -glucuronidase and acid proteinase activity. Elevated enzyme activities have been observed in BAL fluid from rodents exposed to particles. These enzymes may be associated with the breakdown of necrotic tissues.
- 4) Response to oxidant injury as indicated by increased glutathione reductase activity.

The supernatant fluid was analyzed by spectrophotometric, kinetic, and enzymatic analyses for the activities of β -glucuronidase, LDHP, glucose-6-phosphate dehydrogenase, alkaline phosphatase, glutathione reductase, and glutathione peroxidase. Acid proteinase was measured by the release of radiolabeled globin peptides from the trichloroacetic acid-precipitable protein substrate, and total protein was analyzed colorimetrically (Henderson *et al.*, 1985).

Numbers of total nucleated cells recovered in lavage fluid were determined using a cell counter (Coulter Electronic, Hialeah, FL) or a hemocytometer. Cytocentrifuge preparations of resuspended cells were made, stained with Wright's stain (Diff-Quick, Curtin Matheson Scientific, Denver, CO) and the differential cell count determined.

Alveolar macrophages (AM) were recovered from BAL fluid of the same rats as described above. The cells (1×10^6) were suspended in Roswell Park Memorial Institute (RPMI) 1640 culture medium and pelleted by centrifugation and the supernatant RPMI removed. Cells were resuspended in 1 mL of a 1% suspension of IgG antibody-sensitized sheep red blood cells (SRBC) in RPMI 1640. The antibody-sensitized SRBC were made as previously described (Harmsen and Jeska, 1980). The subagglutinating titer of heat-inactivated rabbit anti-SRBC serum was used to sensitize the SRBC. The AM and SRBC suspensions were incubated at 37° C for 1 hour in a humidified atmosphere of 5% CO₂ in air. The AM and SRBC were sedimented by centrifugation and the supernatant discarded. Unphagocytized SRBC were removed by lysing the RBC with water for 30 seconds. Lysing of unphagocytized SRBC was stopped by the addition of an equal volume of saline and cytocentrifuge preparations were made. The slides were stained with Wright's stain (Diff-Quik, American Scientific Products, McGaw Park, IL) and the percent of AM phagocytizing SRBC was determined by light microscopy. Three fields of 100 cells per preparation were counted. Viability was determined by trypan blue exclusion.

Lung Tissue Collagen and Proteinase

In this study, rats sacrificed at 105 weeks of talc exposure were used for collagen metabolism, protein synthesis, and proteinase activity measurements. Tissue and BAL fluid from single rats were used for analyses.

To estimate collagen and protein synthesis, ¹⁴C-proline (0.1 μ Ci/g body weight) was injected intraperitoneally approximately 2 to 3 hours prior to sacrifice. Lung lobes to be analyzed for collagen were frozen in liquid nitrogen and pulverized. The pulverized lungs were extracted overnight in 0.5M acetic acid at 4° C, and centrifuged to separate the insoluble material from the supernatant fluid. The supernatant fluid was separated into high and low molecular weight fractions using Amicon Cones with a size cutoff of approximately 50 kDa.

All samples for collagen analyses from lung and lavage supernatant fluid were hydrolyzed for approximately 18 hours in 6N HCl at 110° C to convert proteins to their individual amino acids, were evaporated to dryness to remove the HCl, and were resuspended in 0.001 N HCl prior to analysis.

Collagen quantity was measured and multiplied by 7.46 to convert BAL or lung tissue hydroxyproline content to BAL or lung tissue collagen content, taking into account that collagen is approximately 13% hydroxyproline by weight (Neuman and Logan, 1950).

Radioactive proline and hydroxyproline were quantitated in the low molecular weight supernatant fluid fraction and in a sample containing both the high molecular weight supernatant fluid fraction and the acetic acid insoluble fraction. Following this, the radioactive proline and hydroxyproline quantities were

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used to calculate the noncollagenous protein synthesis, the collagen production, and the intracellular collagen degradation.

Noncollagenous protein synthesis was measured as the total radioactive proline incorporation into lung tissue minus the incorporation into lung tissue which was related to collagen synthesis. The radioactive proline in collagen was assumed to be equal to the radioactive hydroxyproline, thus, incorporation into collagen was calculated as twice the radioactive hydroxyproline. Collagen production (% of newly synthesized protein that was collagen) was calculated as the percentage of the total incorporation of proline into all proteins constituted by collagen, and adjusted for the 5.4-fold difference in the content of total amino acids (proline and hydroxyproline) between collagen and noncollagenous protein (Pickrell *et al.*, 1987). Intracellular collagen degradation (as a percent of newly synthesized collagen) was calculated as the percentage of total radioactive hydroxyproline in collagen constituted by low molecular weight radioactive hydroxyproline-containing peptides.

Lung tissue proteinase activity was measured as the release of ^{14}C -leucine from prelabeled globin at pH 4.2 and 7.5 (Gregory and Pickrell, 1982; Harkema *et al.*, 1984; Pickrell *et al.*, 1987). Acid proteinase activity was inhibited by leupeptin to indicate either neutrophil and macrophage cathepsin B (inhibited) or macrophage cathepsin D (not inhibited)-like activity. Neutral proteinase activity was inhibited by 1,10-phenanthroline to indicate either macrophage elastase (inhibited) or neutrophil elastase-cathepsin G (not inhibited)-like activity.

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TABLE F1
Number of Rats Evaluated for Lung Talc Burden, Pulmonary Function, and Lung Biochemistry

	Male			Female		
	0 mg/m ³	6 mg/m ³	18 mg/m ³	0 mg/m ³	6 mg/m ³	18 mg/m ³
Lung Burden						
6-Month Interim	- ^a	3	3	-	3	3
11-Month Interim	-	3	3	-	3	3
18-Month Interim	-	3	3	-	2	3
24-Month Interim	-	6	9	-	2	3
Pulmonary Function						
6-Month Interim	9	10	10	10	10	10
11-Month Interim	9	10	10	10	10	10
18-Month Interim	9	10	10	9	9	9
24-Month Interim	3	6	3	6	9	3
Lung Biochemistry						
24-Month Interim	3	6	2	5	9	3

^a Lung burden not measured in 0 mg/m³ rats.

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TABLE F2
Lung Talc Burden (Normalized to Control Lung Weight) of Rats^a

	6 months	12 months	18 months	24 months
Male				
0 mg/m ³	- ^b	-	-	-
6 mg/m ³	2.63 ± 0.24	4.38 ± 0.59	7.31 ± 0.71	10.45 ± 1.26
18 mg/m ³	10.83 ± 0.23	20.96 ± 2.04	27.57 ± 0.91	24.15 ± 3.41
Female				
0 mg/m ³	-	-	-	-
6 mg/m ³	2.43 ± 0.19	4.71 ± 0.26	7.66 ± 0.34	9.10 ± 0.88
18 mg/m ³	8.34 ± 0.12	14.16 ± 3.36	24.33 ± 0.63	29.40 ± 2.40

^a Units are presented as mg talc/g control lung.^b No measurements takenTABLE F3
Lung Talc Burden (Normalized to Exposure Concentration) of Rats^a

	Male		Female	
	6 mg/m ³	18 mg/m ³	6 mg/m ³	18 mg/m ³
6-Month Interim	0.439 ± 0.040	0.602 ± 0.013*	0.406 ± 0.032	0.464 ± 0.007*
12-Month Interim	0.731 ± 0.098	1.165 ± 0.113*	0.785 ± 0.043	0.787 ± 0.187
18-Month Interim	1.22 ± 0.12	1.53 ± 0.05	1.28 ± 0.06	1.35 ± 0.04
24-Month Interim	1.74 ± 0.21	1.34 ± 0.19	1.52 ± 0.15	1.63 ± 0.13

* Significantly different (P≤0.05) from the 6 mg/m³ group by Dunn's or Shirley's test^a Units are presented as mg talc/g control lung/mg/m³

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TABLE F4
Bronchoalveolar Lavage Fluid Enzymes of Rats at the 24-Month Interim Evaluation

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
B-Glucuronidase ^a	1.09 ± 0.40	18.86 ± 3.20*	89.24 ± 14.24**
Lactate dehydrogenase	1,634 ± 545	3,193 ± 606	8,262 ± 380*
Alkaline phosphatase	364.7 ± 147	572.8 ± 86.8	1,604.7 ± 143*
Glutathione reductase	103.03 ± 16.43	99.35 ± 19.79	110.99 ± 51.27
Total protein ^b	1.78 ± 0.40	3.12 ± 0.64	5.79 ± 0.55*
Female			
B-Glucuronidase	3.33 ± 0.97	41.05 ± 4.39**	154.16 ± 17.21**
Lactate dehydrogenase	1,655 ± 266	3,906 ± 444*	14E3 ± 1E3**
Alkaline phosphatase	427.8 ± 30.9	853.6 ± 79.7**	2,504.7 ± 221**
Glutathione reductase	100.6 ± 1.7	135.2 ± 22.4	460.0 ± 44.8*
Total protein	1.20 ± 0.22	4.30 ± 0.36**	12.96 ± 0.28**

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test

** P≤0.01

^a Units presented as mIU/g control lung^b Units presented as mg/g control lung**TABLE F5**
Bronchoalveolar Lavage Fluid Cell Populations of Rats at the 24-Month Interim Evaluation

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Polymorphonuclear cells ^a	0.333 ± 0.167	24.417 ± 2.557*	32.500 ± 3.000*
Lymphocytes	0.000 ± 0.000	0.500 ± 0.258	0.500 ± 0.500
Macrophages	93.67 ± 3.72	70.25 ± 2.53*	62.75 ± 1.75*
Epithelial cells	6.00 ± 3.61	4.83 ± 1.41	4.25 ± 1.75
Female			
Polymorphonuclear cells	0.625 ± 0.315	25.778 ± 2.673**	37.000 ± 1.528**
Lymphocytes	0.000 ± 0.000	0.722 ± 0.188*	1.333 ± 0.667*
Macrophages	91.38 ± 1.75	71.22 ± 2.95**	57.33 ± 4.67**
Epithelial cells	8.00 ± 2.01	2.28 ± 0.50*	4.33 ± 2.60

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test

** P≤0.01

^a Units presented as percent of total cells

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TABLE F6

Viability and Phagocytic Activity of Macrophages in Bronchoalveolar Fluid of Rats at the 24-Month Interim Evaluation

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Viability ^a	63.67 ± 5.91	66.73 ± 1.59	57.70 ± 5.00
Phagocytic activity ^b	83.13 ± 4.54	63.12 ± 8.14	65.30 ^c
Female			
Viability	82.65 ± 9.65	74.64 ± 3.24	61.00 ± 4.42
Phagocytic activity	75.60 ± 5.14	66.51 ± 8.09	70.15 ± 2.85

^a Units are presented as percent viable cells.^b Units are presented as percent cells phagocytizing sheep erythrocytes.^c n=1; no statistic calculated

TABLE F7

Lung Collagen Metabolism and Protein Synthesis in Rats at the 24-Month Interim Evaluation

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Lavage fluid collagenous peptides ^a	39.79 ± 5.07	46.99 ± 6.51	79.21 ± 13.73
Total lung collagen ^b	13.87 ± 0.60	15.98 ± 0.39*	18.88 ± 3.35*
Collagen production ^c	1.58 ± 0.17	1.60 ± 0.17	1.63 ± 0.22
Collagen degradation ^d	31.67 ± 1.72	27.74 ± 1.42	9.18 ± 2.38*
Non-collagenous protein synthesis ^e	142.1 ± 14.5	199.8 ± 22.1*	312.2 ± 10.6**
Female			
Lavage fluid collagenous peptides	78.27 ± 11.64	115.36 ± 8.61*	174.71 ± 13.56**
Total lung collagen	14.32 ± 0.66	19.95 ± 1.58*	36.47 ± 3.39**
Collagen production	0.982 ± 0.185	1.804 ± 0.144*	2.264 ± 0.347**
Collagen degradation	14.41 ± 2.44	21.59 ± 4.99	9.38 ± 1.63
Non-collagenous protein synthesis	173.9 ± 34.5	325.8 ± 90.9	554.3 ± 107*

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as µg/g control lung.^b Units are presented as mg/g control lung.^c Units are presented as percent new protein.^d Units are presented as percent new collagen.^e Units are presented as dpm x 10³/g control lung.

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TABLE F8
Proteinase Activity in Lavage Fluid and Lung Homogenate Supernatant Fluid of Rats
at the 24-Month Interim Evaluation^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Lavage Fluid			
Acid Proteinase	0.994 ± 0.329	1.866 ± 0.174	4.307 ± 0.218*
Cathepsin D	0.147 ± 0.147	0.599 ± 0.150	2.420 ± 0.147**
Cathepsin B	0.924 ± 0.415	1.267 ± 0.094	1.887 ± 0.365
Homogenate Supernatant Fluid			
Acid Proteinase	10.92 ± 0.64	17.51 ± 0.90*	25.13 ± 1.50**
Cathepsin D	8.53 ± 0.91	14.04 ± 0.62*	21.03 ± 1.56**
Cathepsin B	2.39 ± 0.41	3.48 ± 0.37	4.10 ± 0.06*
Neutral Proteinase	0.715 ± 0.168	2.417 ± 0.304*	4.505 ^b
PMN Elastase Cathepsin G	0.490 ± 0.218	1.936 ± 0.242*	4.457 ± 0.377**
Macrophage Elastase Collagenase	0.225 ± 0.099	0.482 ± 0.077	0.000 ^b
Female			
Lavage Fluid			
Acid Proteinase	1.52 ± 0.12	3.46 ± 0.33*	6.05 ± 0.73**
Cathepsin D	0.015 ± 0.015	1.310 ± 0.292*	4.043 ± 0.578**
Cathepsin B	1.61 ± 0.26	2.15 ± 0.22	2.01 ± 0.17
Homogenate Supernatant Fluid			
Acid Proteinase	14.04 ± 0.95	29.43 ± 1.18**	38.61 ± 1.81**
Cathepsin D	10.05 ± 0.68	22.97 ± 1.07**	30.25 ± 1.60**
Cathepsin B	3.99 ± 0.58	6.46 ± 0.60*	8.37 ± 0.42**
Neutral Proteinase	0.648 ± 0.087	5.040 ± 0.418**	12.293 ± 1.598**
PMN Elastase Cathepsin G	0.785 ± 0.142	4.351 ± 0.261**	10.313 ± 2.694**
Macrophage Elastase Collagenase	0.054 ± 0.037	0.683 ± 0.175*	2.012 ± 1.126*

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test

** $P \leq 0.01$

^a Units are presented as mg/hour/mg control lung.

^b n=1; no statistic calculated

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TABLE F9
Respiratory Frequency of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	57.11 ± 0.86	55.00 ± 1.13	54.00 ± 0.75*
11-Month Interim	55.33 ± 1.11	56.10 ± 0.92	53.50 ± 0.99
18-Month Interim	56.50 ± 1.34	55.40 ± 1.08	54.60 ± 1.13
24-Month Interim	57.67 ± 1.20	56.50 ± 1.80	56.67 ± 1.86
Female			
6-Month Interim	52.10 ± 0.55	54.50 ± 1.19	54.30 ± 0.90
11-Month Interim	53.60 ± 0.73	53.70 ± 1.10	55.20 ± 0.94
18-Month Interim	55.44 ± 1.12	54.56 ± 0.93	55.22 ± 1.41
24-Month Interim	57.67 ± 1.23	54.44 ± 0.93	59.00 ± 0.58

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

^a Units are presented as b/min; ratio is (dosed group mean/control group mean)×100TABLE F10
Total Lung Capacity of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	19.86 ± 0.54	19.48 ± 0.46	19.25 ± 0.39
11-Month Interim	20.06 ± 0.32	18.44 ± 0.39**	17.67 ± 0.45**
18-Month Interim	20.30 ± 0.45	18.87 ± 0.41*	16.34 ± 0.52**
24-Month Interim	20.50 ± 0.83	20.20 ± 0.28	16.47 ± 1.53
Female			
6-Month Interim	14.20 ± 0.25	14.56 ± 0.27	13.80 ± 0.27
11-Month Interim	13.29 ± 0.21	12.91 ± 0.17	12.06 ± 0.26**
18-Month Interim	13.94 ± 0.26	12.68 ± 0.28**	11.43 ± 0.31**
24-Month Interim	14.85 ± 0.31	13.73 ± 0.34*	11.50 ± 1.07**

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P=≤.01

^a Units are presented as mL; ratio is (dosed group mean/control group mean)×100

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TABLE F11
Total Lung Capacity/Kilogram Body Weight of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	51.63 ± 1.05	51.45 ± 1.03	53.32 ± 0.78
11-Month Interim	47.71 ± 0.99	44.11 ± 0.87*	43.42 ± 0.74**
18-Month Interim	45.92 ± 1.58	42.98 ± 1.15	38.74 ± 1.50**
24-Month Interim	51.05 ± 4.36	48.49 ± 1.40	44.16 ± 1.29
Female			
6-Month Interim	67.73 ± 1.26	67.06 ± 1.65	65.41 ± 1.50
11-Month Interim	55.21 ± 1.91	52.37 ± 1.05	50.24 ± 1.19
18-Month Interim	45.78 ± 1.26	43.40 ± 1.18	43.26 ± 2.42
24-Month Interim	49.03 ± 1.31	48.93 ± 2.49	44.54 ± 0.51

- * Significantly different (P≤0.05) from the control by Dunn's or Shirley's test
- ** P≤0.01
- ^a Units are presented as mL/kg; ratio is (dosed group mean/control group mean)×100

TABLE F12
Tidal Volume of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	1.83 ± 0.09	1.90 ± 0.08	2.01 ± 0.10
11-Month Interim	1.94 ± 0.06	1.91 ± 0.06	1.93 ± 0.06
18-Month Interim	1.66 ± 0.08	1.63 ± 0.08	1.74 ± 0.08
24-Month Interim	1.50 ± 0.00	1.85 ± 0.16	2.13 ± 0.19*
Female			
6-Month Interim	1.65 ± 0.07	1.53 ± 0.11	1.40 ± 0.07*
11-Month Interim	1.66 ± 0.07	1.68 ± 0.06	1.43 ± 0.09
18-Month Interim	1.54 ± 0.04	1.34 ± 0.06*	1.40 ± 0.03*
24-Month Interim	1.43 ± 0.08	1.39 ± 0.09	1.37 ± 0.15

- * Significantly different (P≤0.05) from the control by Dunn's or Shirley's test
- ^a Units are presented as mL; ratio is (dosed group mean/control group mean)×100

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TABLE F13
Minute Volume of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	102.5 ± 3.9	104.8 ± 4.2	104.8 ± 3.4
11-Month Interim	104.5 ± 3.4	106.2 ± 2.3	100.5 ± 2.8
18-Month Interim	97.34 ± 2.79	90.83 ± 3.45	95.87 ± 4.61
24-Month Interim	92.53 ± 2.64	107.25 ± 6.34	117.77 ± 11.70
Female			
6-Month Interim	85.43 ± 4.22	83.05 ± 4.44	76.36 ± 3.45
11-Month Interim	87.89 ± 3.95	88.18 ± 3.26	78.78 ± 3.81
18-Month Interim	87.14 ± 2.71	73.54 ± 3.02**	76.83 ± 2.29**
24-Month Interim	83.87 ± 5.04	79.64 ± 5.29	82.07 ± 5.95

** Significantly different (P=0.01) from the control by Dunn's or Shirley's test

^a Units are presented as mL/min; ratio is (dosed group mean/control group mean)×100TABLE F14
Minute Volume/Kilogram Body Weight of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	266.0 ± 7.0	277.7 ± 12.8	285.1 ± 9.5
11-Month Interim	247.9 ± 5.9	254.5 ± 7.2	247.6 ± 8.2
18-Month Interim	219.4 ± 4.6	206.8 ± 8.2	226.8 ± 10.5
24-Month Interim	229.5 ± 12.7	256.9 ± 14.8	319.9 ± 38.1
Female			
6-Month Interim	408.7 ± 23.3	381.7 ± 19.5	362.7 ± 19.2
11-Month Interim	365.0 ± 18.9	359.3 ± 18.1	330.1 ± 20.7
18-Month Interim	286.2 ± 11.0	250.6 ± 7.6	291.6 ± 17.7
24-Month Interim	276.9 ± 17.4	282.5 ± 21.4	328.8 ± 57.7

^a Units are presented as mL/min/kg; ratio is (dosed group mean/control group mean)×100

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TABLE F15
Residual Volume of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	2.90 ± 0.21	2.99 ± 0.17	2.64 ± 0.11
11-Month Interim	2.06 ± 0.17	1.63 ± 0.10	1.70 ± 0.16
18-Month Interim	1.96 ± 0.15	1.74 ± 0.13	1.98 ± 0.16
24-Month Interim	3.23 ± 0.48	2.83 ± 0.19	2.20 ± 0.32
Female			
6-Month Interim	2.18 ± 0.14	2.39 ± 0.22	2.47 ± 0.15
11-Month Interim	1.22 ± 0.15	1.25 ± 0.17	1.65 ± 0.14
18-Month Interim	1.28 ± 0.11	1.52 ± 0.13	1.83 ± 0.13**
24-Month Interim	1.68 ± 0.11	1.72 ± 0.23	1.73 ± 0.19

** Significantly different (P≤0.01) from the control by Dunn's or Shirley's test

^a Units are presented as mL; ratio is (dosed group mean/control group mean)×100

TABLE F16
Residual Volume/Total Lung Capacity of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	0.146 ± 0.009	0.154 ± 0.009	0.137 ± 0.004
11-Month Interim	0.102 ± 0.008	0.088 ± 0.005	0.096 ± 0.008
18-Month Interim	0.097 ± 0.007	0.092 ± 0.007	0.121 ± 0.010
24-Month Interim	0.157 ± 0.019	0.140 ± 0.010	0.133 ± 0.011
Female			
6-Month Interim	0.153 ± 0.009	0.163 ± 0.013	0.179 ± 0.011
11-Month Interim	0.091 ± 0.010	0.096 ± 0.013	0.137 ± 0.011*
18-Month Interim	0.091 ± 0.007	0.120 ± 0.010*	0.160 ± 0.009**
24-Month Interim	0.113 ± 0.007	0.125 ± 0.016	0.151 ± 0.005

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mL/mL; ratio is (dosed group mean/control group mean)×100

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Lung Burden, Pulmonary Function, and Lung Biochemistry in Rats

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TABLE F17
Vital Capacity of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	16.96 ± 0.49	16.49 ± 0.44	16.61 ± 0.32
11-Month Interim	18.01 ± 0.27	16.82 ± 0.37*	15.97 ± 0.42**
18-Month Interim	18.35 ± 0.45	17.15 ± 0.38	14.36 ± 0.51**
24-Month Interim	17.27 ± 0.48	17.35 ± 0.34	14.27 ± 1.26
Female			
6-Month Interim	12.02 ± 0.22	12.17 ± 0.20	11.33 ± 0.28
11-Month Interim	12.06 ± 0.20	11.68 ± 0.18	10.40 ± 0.25**
18-Month Interim	12.66 ± 0.21	11.14 ± 0.31**	9.61 ± 0.26**
24-Month Interim	13.15 ± 0.27	11.99 ± 0.32*	9.77 ± 0.90**

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mL; ratio is (dosed group mean/control group mean)×100TABLE F18
Vital Capacity/Total Lung Capacity of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	0.854 ± 0.009	0.846 ± 0.009	0.863 ± 0.004
11-Month Interim	0.898 ± 0.008	0.912 ± 0.005	0.904 ± 0.008
18-Month Interim	0.904 ± 0.007	0.909 ± 0.006	0.878 ± 0.010
24-Month Interim	0.843 ± 0.19	0.859 ± 0.010	0.867 ± 0.011
Female			
6-Month Interim	0.847 ± 0.009	0.837 ± 0.013	0.821 ± 0.011
11-Month Interim	0.908 ± 0.010	0.905 ± 0.012	0.862 ± 0.010*
18-Month Interim	0.908 ± 0.007	0.879 ± 0.010*	0.841 ± 0.009**
24-Month Interim	0.886 ± 0.007	0.874 ± 0.016	0.849 ± 0.005

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mL/mL; ratio is (dosed group mean/control group mean)×100

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Talc, NTP TR 421

TABLE F19
Forced Vital Capacity of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	17.88 ± 0.40	17.15 ± 0.45	17.38 ± 0.41
11-Month Interim	19.03 ± 0.38	18.07 ± 0.43*	17.25 ± 0.45*
18-Month Interim	19.45 ± 0.45	17.92 ± 0.34*	15.28 ± 0.56**
24-Month Interim	17.27 ± 0.61	17.53 ± 0.46	14.90 ± 1.08
Female			
6-Month Interim	12.53 ± 0.33	12.38 ± 0.26	11.27 ± 0.33*
11-Month Interim	12.86 ± 0.25	12.44 ± 0.26	11.22 ± 0.25**
18-Month Interim	13.39 ± 0.24	11.91 ± 0.28**	10.24 ± 0.27**
24-Month Interim	13.08 ± 0.30	12.33 ± 0.33	10.03 ± 0.93**

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mL; ratio is (dosed group mean/control group mean)×100**TABLE F20**
Forced Vital Capacity/Kilogram Body Weight of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	46.48 ± 0.61	45.32 ± 1.18	47.32 ± 1.25
11-Month Interim	45.26 ± 0.95	43.22 ± 0.95	42.42 ± 0.89
18-Month Interim	44.00 ± 1.56	40.82 ± 1.01	36.23 ± 1.57**
24-Month Interim	42.85 ± 2.67	42.00 ± 0.93	40.18 ± 2.32
Female			
6-Month Interim	59.78 ± 1.75	57.01 ± 1.49	53.37 ± 1.48*
11-Month Interim	53.35 ± 1.68	50.43 ± 1.16	46.69 ± 0.90**
18-Month Interim	43.95 ± 1.18	40.76 ± 1.08	38.75 ± 2.17**
24-Month Interim	43.23 ± 1.51	43.87 ± 2.08	38.85 ± 0.48

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mL/kg; ratio is (dosed group mean/control group mean)×100

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Lung Burden, Pulmonary Function, and Lung Biochemistry in Rats

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TABLE F21
Functional Residual Capacity of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	4.48 ± 0.25	4.48 ± 0.22	4.17 ± 0.10
11-Month Interim	3.34 ± 0.24	3.16 ± 0.09	3.19 ± 0.12
18-Month Interim	3.24 ± 0.16	3.07 ± 0.11	3.53 ± 0.14
24-Month Interim	4.53 ± 0.52	3.98 ± 0.24	4.37 ± 0.59
Female			
6-Month Interim	3.51 ± 0.12	3.72 ± 0.16	3.57 ± 0.15
11-Month Interim	2.78 ± 0.12	2.74 ± 0.10	2.87 ± 0.14
18-Month Interim	2.47 ± 0.08	2.82 ± 0.12*	3.17 ± 0.14**
24-Month Interim	3.07 ± 0.13	3.31 ± 0.26	3.27 ± 0.18

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mL; ratio is (dosed group mean/control group mean)×100TABLE F22
Functional Residual Capacity/Total Lung Capacity of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	0.226 ± 0.012	0.230 ± 0.009	0.217 ± 0.006
11-Month Interim	0.166 ± 0.011	0.172 ± 0.006	0.181 ± 0.007
18-Month Interim	0.159 ± 0.006	0.163 ± 0.005	0.217 ± 0.008**
24-Month Interim	0.220 ± 0.020	0.197 ± 0.012	0.268 ± 0.042
Female			
6-Month Interim	0.248 ± 0.008	0.255 ± 0.009	0.258 ± 0.008
11-Month Interim	0.209 ± 0.008	0.212 ± 0.006	0.238 ± 0.010*
18-Month Interim	0.177 ± 0.007	0.223 ± 0.010**	0.277 ± 0.010**
24-Month Interim	0.207 ± 0.009	0.240 ± 0.016	0.287 ± 0.021*

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mL/mL; ratio is (dosed group mean/control group mean)×100

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TABLE F23
Total Pulmonary Resistance of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
18-Month Interim	0.075 ± 0.014	0.096 ± 0.009	0.120 ± 0.009*
24-Month Interim	0.110 ± 0.025	0.087 ± 0.028	0.067 ± 0.020
Female			
18-Month Interim	0.130 ± 0.012	0.131 ± 0.016	0.180 ± 0.010*
24-Month Interim	0.138 ± 0.020	0.131 ± 0.014	0.150 ± 0.035

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

^a Units are presented as cm H₂O/mL/second; ratio is (dosed group mean/control group mean)×100

TABLE F24
Maximum Quasistatic Compliance of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	1.97 ± 0.15	1.84 ± 0.12	2.01 ± 0.13
11-Month Interim	2.32 ± 0.10	1.92 ± 0.12*	1.91 ± 0.09*
18-Month Interim	2.35 ± 0.07	2.09 ± 0.16	1.57 ± 0.07**
24-Month Interim	2.00 ± 0.30	2.01 ± 0.11	1.48 ± 0.20
Female			
6-Month Interim	1.37 ± 0.11	1.47 ± 0.11	1.37 ± 0.08
11-Month Interim	1.273 ± 0.062	1.276 ± 0.033	0.968 ± 0.057**
18-Month Interim	1.704 ± 0.108	1.123 ± 0.050**	0.908 ± 0.068**
24-Month Interim	1.538 ± 0.055	1.263 ± 0.062**	0.883 ± 0.093**

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mL/cm H₂O; ratio is (dosed group mean/control group mean)×100

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Lung Burden, Pulmonary Function, and Lung Biochemistry in Rats

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TABLE F25
Quasistatic Chord Compliance of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	1.18 ± 0.05	1.16 ± 0.04	1.17 ± 0.03
11-Month Interim	1.34 ± 0.02	1.20 ± 0.04*	1.15 ± 0.04**
18-Month Interim	1.343 ± 0.037	1.205 ± 0.040*	0.982 ± 0.037**
24-Month Interim	1.167 ± 0.104	1.220 ± 0.035	0.890 ± 0.124
Female			
6-Month Interim	0.824 ± 0.030	0.895 ± 0.091	0.802 ± 0.024
11-Month Interim	0.841 ± 0.020	0.809 ± 0.016	0.684 ± 0.025**
18-Month Interim	0.879 ± 0.019	0.749 ± 0.027**	0.607 ± 0.030**
24-Month Interim	0.883 ± 0.035	0.764 ± 0.024*	0.573 ± 0.084**

* Significantly different ($P \leq 0.05$) from the control by Dunn's or Shirley's test

** $P \leq 0.01$

^a Units are presented as mL/cm H₂O; ratio is (dosed group mean/control group mean)×100

TABLE F26
Dynamic Compliance of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	0.546 ± 0.053	0.575 ± 0.043	0.536 ± 0.058
11-Month Interim	0.748 ± 0.041	0.647 ± 0.048	0.687 ± 0.046
18-Month Interim	0.990 ± 0.080	0.741 ± 0.043*	0.685 ± 0.050**
24-Month Interim	0.930 ± 0.173	0.987 ± 0.130	1.173 ± 0.186
Female			
6-Month Interim	0.399 ± 0.029	0.445 ± 0.032	0.380 ± 0.034
11-Month Interim	0.492 ± 0.024	0.426 ± 0.027*	0.393 ± 0.020**
18-Month Interim	0.618 ± 0.053	0.527 ± 0.027	0.372 ± 0.025**
24-Month Interim	0.650 ± 0.065	0.618 ± 0.045	0.377 ± 0.077*

* Significantly different ($P \leq 0.05$) from the control by Dunn's or Shirley's test

** $P \leq 0.01$

^a Units are presented as mL/cm H₂O; ratio is (dosed group mean/control group mean)×100

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TABLE F27
Peak Expiratory Flow of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	139.9 ± 1.9	138.7 ± 2.8	132.5 ± 4.0
11-Month Interim	136.6 ± 1.4	133.5 ± 4.5	132.9 ± 2.0
18-Month Interim	132.2 ± 1.2	132.3 ± 0.7	129.5 ± 0.6**
24-Month Interim	126.1 ± 2.7	124.5 ± 1.9	124.0 ± 1.0
Female			
6-Month Interim	120.1 ± 8.7	122.3 ± 6.6	113.5 ± 5.7
11-Month Interim	125.3 ± 4.3	123.9 ± 4.9	123.2 ± 2.1
18-Month Interim	120.6 ± 3.0	113.2 ± 2.3	114.3 ± 2.5
24-Month Interim	117.1 ± 2.5	116.7 ± 3.4	110.1 ± 4.7

** Significantly different (P≤0.01) from the control by Dunn's or Shirley's test

^a Units are presented as mL/second; ratio is (dosed group mean/control group mean)×100TABLE F28
Peak Expiratory Flow/Forced Vital Capacity of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	7.85 ± 0.18	8.12 ± 0.22	7.63 ± 0.16
11-Month Interim	7.21 ± 0.18	7.44 ± 0.33	7.74 ± 0.20
18-Month Interim	6.82 ± 0.15	7.40 ± 0.14*	8.57 ± 0.29**
24-Month Interim	7.31 ± 0.14	7.13 ± 0.21	8.40 ± 0.52
Female			
6-Month Interim	9.56 ± 0.62	9.82 ± 0.35	10.08 ± 0.47
11-Month Interim	9.73 ± 0.22	9.95 ± 0.31	11.01 ± 0.23**
18-Month Interim	9.02 ± 0.20	9.57 ± 0.37	11.21 ± 0.32**
24-Month Interim	8.96 ± 0.22	9.47 ± 0.19	11.16 ± 1.13**

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mL/second/mL; ratio is (dosed group mean/control group mean)×100

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Lung Burden, Pulmonary Function, and Lung Biochemistry in Rats

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TABLE F29
Expiratory Flow 10% Forced Vital Capacity of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	28.22 ± 2.04	24.20 ± 1.77	19.60 ± 2.67*
11-Month Interim	26.33 ± 1.82	20.80 ± 1.14*	21.60 ± 1.50
18-Month Interim	19.00 ± 1.87	18.00 ± 1.61	20.70 ± 1.17
24-Month Interim	11.33 ± 1.20	18.67 ± 1.50	18.33 ± 1.76
Female			
6-Month Interim	17.40 ± 2.88	18.10 ± 3.10	16.60 ± 2.68
11-Month Interim	19.20 ± 2.36	19.50 ± 1.97	23.30 ± 2.29
18-Month Interim	19.67 ± 1.62	19.00 ± 1.45	21.78 ± 0.66
24-Month Interim	12.67 ± 1.65	18.44 ± 1.51*	17.00 ± 2.52

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

* Units are presented as mL/second; ratio is (dosed group mean/control group mean)×100

TABLE F30
Expiratory Flow 10% Forced Vital Capacity/Forced Vital Capacity of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	1.58 ± 0.11	1.41 ± 0.09	1.13 ± 0.16*
11-Month Interim	1.39 ± 0.10	1.16 ± 0.08	1.27 ± 0.11
18-Month Interim	0.986 ± 0.106	1.002 ± 0.085	1.372 ± 0.085*
24-Month Interim	0.661 ± 0.085	1.057 ± 0.065*	1.256 ± 0.188*
Female			
6-Month Interim	1.37 ± 0.21	1.43 ± 0.23	1.45 ± 0.22
11-Month Interim	1.47 ± 0.17	1.55 ± 0.14	2.07 ± 0.19**
18-Month Interim	1.47 ± 0.13	1.62 ± 0.15	2.14 ± 0.09**
24-Month Interim	0.959 ± 0.109	1.488 ± 0.102*	1.693 ± 0.170*

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

* Units are presented as mL/second/mL; ratio is (dosed group mean/control group mean)×100

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TABLE F31
Expiratory Flow 25% Forced Vital Capacity of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	63.56 ± 3.30	55.00 ± 5.57	44.30 ± 6.59*
11-Month Interim	62.00 ± 2.89	60.40 ± 3.46	59.30 ± 3.36
18-Month Interim	50.50 ± 2.57	54.20 ± 2.45	62.20 ± 2.80**
24-Month Interim	47.00 ± 2.89	51.33 ± 3.97	60.00 ± 3.79
Female			
6-Month Interim	44.30 ± 7.73	41.20 ± 7.14	35.60 ± 5.59
11-Month Interim	50.40 ± 5.68	43.00 ± 5.69	54.60 ± 4.01
18-Month Interim	52.33 ± 4.57	42.56 ± 4.76	49.00 ± 3.67
24-Month Interim	40.67 ± 3.80	49.33 ± 6.17	46.00 ± 12.49

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mL/second; ratio is (dosed group mean/control group mean)×100

TABLE F32
Expiratory Flow 25% Forced Vital Capacity/Forced Vital Capacity of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	3.56 ± 0.19	3.21 ± 0.31	2.54 ± 0.37
11-Month Interim	3.26 ± 0.14	3.35 ± 0.20	3.47 ± 0.24
18-Month Interim	2.61 ± 0.15	3.03 ± 0.14*	4.14 ± 0.27**
24-Month Interim	2.72 ± 0.07	2.92 ± 0.20	4.06 ± 0.35*
Female			
6-Month Interim	3.50 ± 0.59	3.25 ± 0.53	3.10 ± 0.43
11-Month Interim	3.88 ± 0.42	3.43 ± 0.44	4.88 ± 0.37
18-Month Interim	3.91 ± 0.34	3.60 ± 0.44	4.75 ± 0.27
24-Month Interim	3.10 ± 0.24	3.95 ± 0.44	4.66 ± 1.28

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mL/second/mL; ratio is (dosed group mean/control group mean)×100

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TABLE F33
Expiratory Flow 50% Forced Vital Capacity of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	111.33 ± 7.11	94.00 ± 7.61	78.70 ± 10.05*
11-Month Interim	111.7 ± 4.4	100.1 ± 7.1	102.1 ± 6.2
18-Month Interim	98.75 ± 6.00	97.10 ± 3.59	107.70 ± 5.25
24-Month Interim	99.33 ± 10.17	92.33 ± 4.47	94.67 ± 9.02
Female			
6-Month Interim	75.30 ± 11.98	73.90 ± 10.54	66.00 ± 8.52
11-Month Interim	85.50 ± 8.87	78.00 ± 10.09	94.10 ± 5.57
18-Month Interim	93.00 ± 8.40	76.11 ± 9.60	87.67 ± 6.91
24-Month Interim	86.50 ± 7.12	85.89 ± 10.40	83.67 ± 23.90

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

^a Units are presented as mL/second; ratio is (dosed group mean/control group mean)×100TABLE F34
Expiratory Flow 50% Forced Vital Capacity/Forced Vital Capacity of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	6.23 ± 0.39	5.50 ± 0.47	4.49 ± 0.55*
11-Month Interim	5.86 ± 0.18	5.55 ± 0.40	5.95 ± 0.40
18-Month Interim	5.08 ± 0.30	5.43 ± 0.21	7.18 ± 0.50**
24-Month Interim	5.73 ± 0.44	5.30 ± 0.36	6.38 ± 0.62
Female			
6-Month Interim	5.95 ± 0.90	5.85 ± 0.77	5.79 ± 0.67
11-Month Interim	6.58 ± 0.62	6.21 ± 0.77	8.39 ± 0.48*
18-Month Interim	6.92 ± 0.59	6.48 ± 0.90	8.49 ± 0.54
24-Month Interim	6.63 ± 0.55	6.88 ± 0.77	8.50 ± 2.50

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mL/second/mL; ratio is (dosed group mean/control group mean)×100

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TABLE F35
Mean Midexpiratory Flow of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	101.90 ± 5.20	89.30 ± 7.34	74.27 ± 9.38*
11-Month Interim	102.52 ± 3.54	94.92 ± 6.02	94.11 ± 4.51
18-Month Interim	93.12 ± 3.99	91.41 ± 2.81	98.44 ± 3.67
24-Month Interim	87.13 ± 6.27	87.78 ± 3.74	90.33 ± 7.07
Female			
6-Month Interim	71.07 ± 12.01	70.72 ± 10.66	60.65 ± 7.99
11-Month Interim	81.38 ± 7.94	73.24 ± 9.19	87.91 ± 5.04
18-Month Interim	85.98 ± 6.80	69.51 ± 7.53	81.79 ± 5.58
24-Month Interim	78.28 ± 5.27	79.94 ± 9.44	75.13 ± 19.66

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

^a Units are presented as mL/second; ratio is (dosed group mean/control group mean)×100

TABLE F36
Mean Midexpiratory Flow/Forced Vital Capacity of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	5.71 ± 0.30	5.23 ± 0.44	4.24 ± 0.51
11-Month Interim	5.39 ± 0.16	5.27 ± 0.35	5.49 ± 0.32
18-Month Interim	4.78 ± 0.13	5.11 ± 0.18	6.55 ± 0.39**
24-Month Interim	5.04 ± 0.24	5.03 ± 0.29	6.10 ± 0.56
Female			
6-Month Interim	5.62 ± 0.91	5.59 ± 0.78	5.31 ± 0.62
11-Month Interim	6.27 ± 0.56	5.83 ± 0.70	7.85 ± 0.45*
18-Month Interim	6.41 ± 0.48	5.90 ± 0.72	7.94 ± 0.41
24-Month Interim	5.99 ± 0.39	6.40 ± 0.69	7.65 ± 2.13

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mL/second/mL; ratio is (dosed group mean/control group mean)×100

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Lung Burden, Pulmonary Function, and Lung Biochemistry in Rats

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TABLE F37
Carbon Monoxide Diffusing Capacity of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	0.364 ± 0.014	0.347 ± 0.008	0.336 ± 0.010
11-Month Interim	0.400 ± 0.010	0.373 ± 0.010	0.331 ± 0.020**
18-Month Interim	0.338 ± 0.022	0.301 ± 0.015	0.235 ± 0.009**
24-Month Interim	0.303 ± 0.027	0.288 ± 0.011	0.177 ± 0.035*
Female			
6-Month Interim	0.238 ± 0.012	0.241 ± 0.008	0.213 ± 0.010
11-Month Interim	0.233 ± 0.008	0.231 ± 0.005	0.190 ± 0.003**
18-Month Interim	0.233 ± 0.010	0.207 ± 0.009	0.137 ± 0.011**
24-Month Interim	0.198 ± 0.007	0.183 ± 0.006	0.113 ± 0.017**

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mL/minute/mm Hg; ratio is (dosed group mean/control group mean)×100TABLE F38
Carbon Monoxide Diffusing Capacity/Lung Volume of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	0.020 ± 0.001	0.020 ± 0.000	0.019 ± 0.000
11-Month Interim	0.021 ± 0.000	0.021 ± 0.001	0.019 ± 0.001*
18-Month Interim	0.017 ± 0.001	0.025 ± 0.008	0.014 ± 0.001*
24-Month Interim	0.015 ± 0.002	0.015 ± 0.001	0.010 ± 0.002*
Female			
6-Month Interim	0.019 ± 0.001	0.019 ± 0.001	0.017 ± 0.001
11-Month Interim	0.018 ± 0.001	0.019 ± 0.000	0.017 ± 0.000*
18-Month Interim	0.017 ± 0.001	0.016 ± 0.001	0.012 ± 0.001**
24-Month Interim	0.013 ± 0.001	0.013 ± 0.001	0.009 ± 0.001

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Ratio is (dosed group mean/control group mean)×100

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TABLE F39
Carbon Monoxide Diffusing Capacity/Kilogram Body Weight of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	0.949 ± 0.039	0.917 ± 0.017	0.914 ± 0.026
11-Month Interim	0.951 ± 0.021	0.892 ± 0.021	0.821 ± 0.043**
18-Month Interim	0.759 ± 0.043	0.683 ± 0.029	0.554 ± 0.016**
24-Month Interim	0.749 ± 0.056	0.691 ± 0.025	0.465 ± 0.062*
Female			
6-Month Interim	1.13 ± 0.05	1.11 ± 0.04	1.01 ± 0.04
11-Month Interim	0.968 ± 0.045	0.939 ± 0.033	0.792 ± 0.019**
18-Month Interim	0.766 ± 0.034	0.705 ± 0.028	0.502 ± 0.028**
24-Month Interim	0.656 ± 0.031	0.650 ± 0.027	0.435 ± 0.036*

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mL/minute/mm Hg/kg; ratio is (dosed group mean/control group mean)×100

TABLE F40
Percent Forced Vital Capacity Expired in 0.1 Second of Rats^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	61.11 ± 1.52	59.80 ± 2.15	53.90 ± 2.64
11-Month Interim	58.22 ± 0.98	57.40 ± 2.74	60.30 ± 1.63
18-Month Interim	55.00 ± 0.63	58.30 ± 0.90*	66.50 ± 2.13**
24-Month Interim	58.67 ± 1.20	57.00 ± 1.71	64.00 ± 2.89
Female			
6-Month Interim	62.80 ± 5.17	64.20 ± 3.82	63.40 ± 3.86
11-Month Interim	67.00 ± 2.82	65.20 ± 3.61	75.50 ± 1.78*
18-Month Interim	66.44 ± 2.60	64.56 ± 3.57	75.78 ± 1.56*
24-Month Interim	65.83 ± 2.09	66.78 ± 3.31	73.00 ± 9.17

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as percent forced vital capacity; ratio is (dosed group mean/control group mean)×100

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Lung Burden, Pulmonary Function, and Lung Biochemistry in Rats

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TABLE F41
Slope III of N₂ Washout of Rats¹

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
6-Month Interim	0.400 ± 0.023	0.431 ± 0.037	0.481 ± 0.049
11-Month Interim	0.449 ± 0.019	0.446 ± 0.037	0.437 ± 0.040
18-Month Interim	0.393 ± 0.037	0.361 ± 0.035	0.555 ± 0.041*
24-Month Interim	0.627 ± 0.077	0.438 ± 0.045	0.597 ± 0.083
Female			
6-Month Interim	0.587 ± 0.059	0.528 ± 0.049	0.596 ± 0.042
11-Month Interim	0.704 ± 0.027	0.735 ± 0.029	0.813 ± 0.076
18-Month Interim	0.601 ± 0.053	0.699 ± 0.074	1.008 ± 0.087**
24-Month Interim	0.535 ± 0.040	0.580 ± 0.071	1.520 ± 0.409*

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

¹ Units are presented as percent N₂/mL; ratio is (dosed group mean/control group mean)×100

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APPENDIX G

LUNG BURDEN AND LUNG BIOCHEMISTRY IN MICE

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METHODS

Lung Burden

Lung talc burden was measured to determine the relationship between the exposure concentration and the amount of talc deposited and retained within the pulmonary region of the respiratory tract. The method used for determination of talc in the lungs of rats and mice has been published (Hanson *et al.*, 1985). Lung burdens of talc were determined on the left lung of 4 male and 4 female mice from each exposure group sacrificed at 27, 52, and 79 weeks after the start of exposure. At 103 to 104 weeks, lung burdens were determined on the left lungs of two mice from the biochemistry group. The analysis was based on determination of acid insoluble magnesium in the lung. MRI reported that the value for the magnesium was 19.33% for batch 02, and 19.47% for batch 03. The values reported by MRI and the results of the analysis at LITRI were close to the theoretical value of magnesium for talc (19.22%). Since mice sacrificed at 27, 52, and 79 weeks had been exposed to only batch 02 of talc, 19.33% magnesium was used to calculate quantity of talc for these mice. Since batch 03 was used for the last 4 months of exposure, and lung burdens of mice after 103 to 104 weeks of exposure talc would be expected to contain substantial amounts of batch 03 talc, 19.47% magnesium was used to calculate quantity of talc in lungs for these mice.

All operations in conjunction with the tissue analysis for talc were done with talc-free gloves. Left lung lobes were weighed at necropsy and stored frozen (-20° C) until used. Lungs were homogenized using water and the proteins precipitated with 70% perchloric acid. The individual samples were filtered and washed with 5% trichloroacetic acid (TCA) to remove perchlorates. Washing continued until magnesium levels in the wash were within 10% of levels in the TCA solution (≤ 0.03 ppm magnesium). Filters and tissue residues were placed in 15 mL porcelain crucibles, dried slowly (200° C), and then ashed at 600° C for 1 hour. Ashed samples were transferred to Teflon beakers using 2 mL HCl and evaporated to dryness. Samples were digested in hydrofluoric acid (HF), and the HF evaporated. Additional HF was added and reevaporated. Sulfuric acid was added to remove trace HF, and samples were diluted with distilled water and analyzed for magnesium by atomic absorbance (Perkin Elmer, Model 306, Atomic Absorption Spectrophotometer) with a magnesium hollow cathode lamp and an air acetylene flame (Hanson *et al.*, 1985).

Lung Biochemistry

In this study, bronchoalveolar lavage (BAL) fluid enzyme activity and cell numbers were measured as biochemical and cytological indicators of pulmonary injury from inhalation of talc. Four mice of each sex from each exposure group were sacrificed at 27, 52, and 79 weeks, and all remaining lung toxicology mice were sacrificed at 103 to 104 weeks. Numbers of animals at each sacrifice are shown below.

Mice were anesthetized with halothane and sacrificed by exsanguination from the abdominal aorta or renal artery. The heart and lung block were removed. Mice were given endobronchial saline lavage (3 to 4 mL total volume in four, 0.75 to 1.0 mL washes) and the BAL fluid centrifuged at $300 \times G$ to separate the cells from the supernatant fluid.

At all sacrifices, biochemical analyses were done on lavage fluid from single mice. At the 103 to 104 week terminal sacrifice where lung burden measurements were also performed on the left lung lobes, mouse lavage fluids were paired (from 2 mice) to obtain sufficient cells for the analyses and paired mouse lung tissue samples (from 2 mice) were analyzed to obtain sufficient lung tissue for collagen analyses.

Airway Fluid Enzymes and Cytology

In this study, BAL fluid was analyzed to determine degree of:

- 1) Cell injury as indicated by quantities of BAL fluid lactate dehydrogenase (LDH).
- 2) Chronic inflammatory response as indicated by presence of increased numbers of polymorphonuclear leukocytes (PMN) and pulmonary alveolar macrophages (AM) as well as increased BAL fluid protein and alkaline phosphatase activity.

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- 3) Lysosomal activation as indicated by quantities of BAL fluid β -glucuronidase and acid proteinase. Elevated quantities of these enzymes have been observed in BAL fluid from rodents exposed to particulates. These enzymes may be associated with the breakdown of necrotic tissues.
- 4) Response to oxidant injury as indicated by increased quantities of glutathione reductase and peroxidase activity.

The supernatant fluid was analyzed for the activities of β -glucuronidase, LDH, glucose-6-phosphate dehydrogenase, alkaline phosphatase, glutathione reductase, and glutathione peroxidase by spectrophotometric, kinetic, and enzymatic techniques. Acid proteinase was measured by release of radiolabeled globin from the trichloroacetic acid precipitable protein substrate, and total protein was analyzed colorimetrically (Henderson *et al.*, 1985). β -Glucuronidase was not performed at 27-week interim evaluation, but was performed at all other sacrifice times.

Numbers of total nucleated cells recovered in lavage fluid were determined on each sample using a cell counter (Coulter Electronics, Hialeah, FL) or a hemocytometer. Cytocentrifuge preparations of resuspended cells were made, stained with Wright's stain (Diff-Quik, Curtin Matheson Scientific, Denver, CO) and differential cell counts were determined. At the 27, 52, and 79 week interim sacrifices, analyses were done on individual mice.

Alveolar macrophages (AM) were recovered from BAL fluid of the same mice as described above. Cells (0.5×10^6) in Roswell Park Memorial Institute (RPMI) culture medium were pelleted by centrifugation and the supernatant RPMI removed. Cells were resuspended in 1 mL of a 1% suspension of IgG antibody-sensitized sheep red blood cells (SRBC) in RPMI 1640. The antibody sensitized SRBC were made as previously described (Harmsen and Jeska, 1980). The subagglutinating titer of heat-inactivated rabbit anti-SRBC serum was used to sensitize the SRBC. The AM and SRBC suspensions were incubated at 37° C for 1 hour in a humidified atmosphere of 5% CO₂ in air. The AM and SRBC were sedimented by centrifugation and the supernatant discarded. Unphagocytized SRBC were removed by lysing the RBC with water for 30 seconds. The lysing of unphagocytized SRBC was stopped by the addition of an equal volume of saline and cytocentrifuge preparations were made. The slides were stained with a rapid Wright's stain (Diff-Quik, American Scientific Products, McGaw Park, IL) and the number of AM phagocytizing 0, 1, 2, 3 to 4, and > 4 SRBC was determined by light microscopy. Three fields of 100 cells per preparation were counted. Viability of macrophages was not determined at the 27, 52, and 79 week sacrifices because the small number of cells recovered from these mice lungs precluded the measurement of cell viability. Viability determination of macrophages was made on macrophages obtained at the final sacrifice because sufficient numbers of cells were generally available at this time.

Lung Tissue Collagen and Proteinase

At 27-, 52-, and 79-week sacrifices, collagen content of lungs and lavage fluid was measured. At the 103 to 104 week sacrifice, additional collagen metabolism and protein synthesis measurements were made on survivors from each group. Proteinase activities were measured at all sacrifice times.

The supernatant BAL fluid was analyzed for hydroxyproline and acid proteinase. Lung tissue and bronchoalveolar lavage (BAL) fluid samples were hydrolyzed with 6N HCl at 110° C for approximately 18 hours to convert proteins to their individual amino acids. Collagen quantity was measured and multiplied by 7.46 to convert BAL or lung tissue hydroxyproline content to BAL or lung tissue collagen content, taking into account that collagen is approximately 13% hydroxyproline by weight (Neuman and Logan, 1950).

Additional collagen metabolism measurements were made on the mice sacrificed after 103 to 104 weeks of talc exposure to further define collagen metabolism. Approximately 2 to 3 hours prior to sacrifice, ¹⁴C-proline (0.1 μ Ci/g body weight) was injected intraperitoneally to estimate collagen and protein synthesis. Radioactive proline and hydroxyproline were quantitated in lung hydrolysate. Following this,

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the radioactive proline and hydroxyproline quantities were used to calculate the noncollagenous protein synthesis, and the collagen production.

Noncollagenous protein synthesis was indicated as total ^{14}C -proline incorporation into lung tissue minus the incorporation into lung tissue which was related to collagen synthesis. The radioactive proline in collagen was assumed to be equal to the radioactive hydroxyproline, thus, incorporation into collagen was calculated as twice the radioactive hydroxyproline. Collagen production (% of newly synthesized protein that was collagen) was calculated as the percent incorporation of proline into collagen constituted of the total incorporation of proline into all proteins, and adjusted for the 5.4-fold difference in the content of total amino acids (proline and hydroxyproline) between collagen and noncollagenous protein (Pickrell *et al.*, 1987).

At each sacrifice time, lung tissue proteinase activity was measured as the release of ^{14}C -leucine from prelabeled globin at pH 4.2 and 7.5 (Gregory and Pickrell, 1982; Harkema *et al.*, 1984; Pickrell *et al.*, 1987). Acid proteinase activity was inhibited by leupeptin to indicate either cathepsin B (inhibited) or cathepsin D (not inhibited)-like activity. Neutral proteinase activity was inhibited by 1, 10-phenanthroline to indicate either macrophage elastase (inhibited) or neutrophil elastase-cathepsin G (not inhibited)-like activity.

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Lung Burden and Lung Biochemistry of Mice

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TABLE G1
Number of Mice Evaluated for Lung Talc Burden and Lung Biochemistry

	Male			Female		
	0 mg/m ³	6 mg/m ³	18 mg/m ³	0 mg/m ³	6 mg/m ³	18 mg/m ³
Lung Burden						
6-Month Interim	— ^a	2	4	—	4	4
12-Month Interim	—	4	4	—	4	4
18-Month Interim	—	2	1	—	4	3
24-Month Interim	—	8	6	—	6	5
Lung Biochemistry						
6-Month Interim	4	4	4	4	4	4
12-Month Interim	4	4	4	4	4	4
18-Month Interim	4	4	4	4	4	4
24-Month Interim	9	8	6	7	6	5

^a Lung burden not measured in 0 mg/m³ mice

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TABLE G2
Lung Talc Burden (Normalized to Control Lung Weight) of Mice^a

	6 months	12 months	18 months	24 months
Male				
0 mg/m ³	- ^b	-	-	-
6 mg/m ³	0.415 ± 0.114	1.084 ± 0.130	0.426 ± 0.040	2.973 ± 0.762
18 mg/m ³	1.41 ± 0.29	9.00 ± 1.45	8.36	19.73 ± 4.03
Female				
0 mg/m ³	-	-	-	-
6 mg/m ³	0.524 ± 0.056	0.707 ± 0.170	1.387 ± 0.178	2.667 ± 0.720
18 mg/m ³	1.35 ± 0.24	6.17 ± 1.39	7.83 ± 1.36	20.05 ± 0.98

^a Units are presented as mg talc/g control lung.

^b Not examined

TABLE G3
Lung Talc Burden (Normalized to Exposure Concentration) of Mice^a

	Male		Female	
	6 mg/m ³	18 mg/m ³	6 mg/m ³	18 mg/m ³
6-Month Interim	0.069 ± 0.019	0.078 ± 0.016	0.087 ± 0.009	0.075 ± 0.013
12-Month Interim	0.181 ± 0.022	0.500 ± 0.081 ^a	0.118 ± 0.028	0.343 ± 0.077 ^a
18-Month Interim	0.071 ± 0.007	0.464 ^b	0.231 ± 0.030	0.435 ± 0.075
24-Month Interim	0.496 ± 0.127	1.096 ± 0.224 ^a	0.445 ± 0.120	1.114 ± 0.055 ^a

^a Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test

^a Units are presented as mg talc/g control lung/mg/m³

^b n=1; no statistic calculated

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TABLE G4
Bronchoalveolar Lavage Fluid Enzymes of Mice at the 6-Month Interim Evaluation

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Lactate dehydrogenase ^a	1,408 ± 658	1,317 ± 106	2,107 ± 336
Glutathione reductase	148.4 ± 33.8	123.3 ± 28.3	227.2 ± 65.6
Total protein ^b	3.57 ± 0.89	1.92 ± 0.70	6.24 ± 1.23
Female			
Lactate dehydrogenase	1,988 ± 157	2,351 ± 180	1,400 ± 197
Glutathione reductase	206.8 ± 14.7	166.0 ± 21.3	148.5 ± 29.4
Total protein	2.55 ± 0.53	4.43 ± 0.34	6.89 ± 4.29

^a Units are presented as mIU/g control lung.

^b Units are presented as mg/g controls lung.

TABLE G5
Bronchoalveolar Lavage Fluid Enzymes of Mice at the 12-Month Interim Evaluation

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
β-Glucuronidase ^a	0.188 ± 0.114	0.486 ± 0.346	12.787 ± 3.604*
Lactate dehydrogenase	1,107.6 ± 545	540.2 ± 59.0	1,487.1 ± 456
Glutathione reductase	89.50 ± 11.65	91.67 ± 6.60	302.40 ± 65.15*
Total protein ^b	2.21 ± 0.74	1.56 ± 0.33	6.19 ± 2.63
Female			
β-Glucuronidase	0.073 ± 0.073	0.413 ± 0.251	9.786 ± 2.271**
Lactate dehydrogenase	1,209.7 ± 305	447.5 ± 76.1	1,805.3 ± 285
Glutathione reductase	113.57 ± 19.78	97.93 ± 14.93	198.65 ± 23.44
Total protein	3.54 ± 1.27	3.61 ± 1.38	4.82 ± 2.88

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mIU/g control lung.

^b Units are presented as mg/g control lung.

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TABLE G6
Bronchoalveolar Lavage Fluid Enzymes of Mice at the 18-Month Interim Evaluation

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
β -Glucuronidase ^a	0.000 \pm 0.000	1.344 \pm 1.267	9.937 \pm 4.196**
Lactate dehydrogenase	434.0 \pm 45.7	642.4 \pm 119	1,039.9 \pm 168**
Glutathione reductase	63.93 \pm 14.16	106.38 \pm 12.15	217.18 \pm 45.29*
Total protein ^b	3.43 \pm 0.62	6.23 \pm 0.97*	9.45 \pm 1.95**
Female			
β -Glucuronidase	4.243 \pm 4.203	0.334 \pm 0.334	19.064 \pm 9.200
Lactate dehydrogenase	501.4 \pm 46.9	404.2 \pm 97.6	1,217.6 \pm 255*
Glutathione reductase	73.19 \pm 14.94	71.27 \pm 12.11	240.55 \pm 44.06*
Total protein	2.96 \pm 0.40	3.41 \pm 0.92	9.59 \pm 1.23*

* Significantly different (P \leq 0.05) from the control group by Dunn's or Shirley's test** P \leq 0.01^a Units are presented as mIU/g control lung.^b Units are presented as mg/g control lung.

TABLE G7
Bronchoalveolar Lavage Fluid Enzymes of Mice at the 24-Month Interim Evaluation

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
β -Glucuronidase ^a	0.000 \pm 0.000	1.811 \pm 0.878**	16.571 \pm 3.932**
Lactate dehydrogenase	1,769 \pm 259	1,439 \pm 295	2,965 \pm 131*
Glutathione reductase	73.66 \pm 9.75	87.55 \pm 25.16	229.53 \pm 58.46*
Total protein ^b	1.69 \pm 0.20	2.34 \pm 0.22	4.68 \pm 0.70**
Female			
β -Glucuronidase	0.000 \pm 0.000	2.624 \pm 1.176**	13.778 \pm 2.640**
Lactate dehydrogenase	1,082 \pm 155	1,596 \pm 197*	2,026 \pm 279**
Glutathione reductase	68.66 \pm 7.42	73.37 \pm 13.91	163.46 \pm 33.43*
Total protein	1.111 \pm 0.310	0.872 \pm 0.261	2.228 \pm 0.501

* Significantly different (P \leq 0.05) from the control group by Dunn's or Shirley's test** P \leq 0.01^a Units are presented as mIU/g control lung.^b Units are presented as mg/g control lung.

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TABLE G8
Bronchoalveolar Lavage Fluid Cell Populations of Mice at the 6-Month Interim Evaluation^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Polymorphonucleated cells	0.250 ± 0.250	3.250 ± 1.250	12.000 ± 3.764**
Lymphocytes	0.750 ± 0.750	0.750 ± 0.479	0.000 ± 0.000
Macrophages	92.50 ± 3.23	95.75 ± 1.44	84.75 ± 2.95
Epithelial cells	6.500 ± 3.775	0.250 ± 0.250	3.250 ± 1.250
Female			
Polymorphonuclear cells	0.000 ± 0.000	1.250 ± 0.629*	1.750 ± 0.854**
Lymphocytes	0.000 ± 0.000	1.000 ± 1.000	0.000 ± 0.000
Macrophages	95.00 ± 2.16	94.75 ± 1.44	96.00 ± 1.22
Epithelial cells	5.00 ± 2.16	3.00 ± 1.73	2.25 ± 1.31

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as percent of total cells.

TABLE G9
Bronchoalveolar Lavage Fluid Cell Populations of Mice at the 12-Month Interim Evaluation^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Polymorphonuclear cells	26.75 ± 15.12	7.50 ± 5.85	15.00 ± 14.01
Lymphocytes	0.750 ± 0.250	2.250 ± 1.436	0.333 ± 0.333
Macrophages	70.50 ± 14.56	83.25 ± 6.91	73.33 ± 12.14
Epithelial cells	2.00 ± 1.41	7.00 ± 2.12	11.33 ± 7.36
Female			
Polymorphonuclear cells	1.33 ± 1.33	34.50 ± 10.27*	2.25 ± 0.85
Lymphocytes	1.000 ± 0.577	3.500 ± 1.500	0.000 ± 0.000
Macrophages	92.67 ± 0.33	58.25 ± 11.65	91.00 ± 2.04
Epithelial cells	5.00 ± 1.53	3.75 ± 1.75	6.75 ± 2.84

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test

^a Units are presented as percent of total cells.

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TABLE G10
Bronchoalveolar Lavage Fluid Cell Populations of Mice at the 18-Month Interim Evaluation^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Polymorphonuclear cells	0.250 ± 0.250	8.750 ± 4.404	19.000 ± 6.258*
Lymphocytes	0.000 ± 0.000	0.500 ± 0.500	1.000 ± 0.577
Macrophages	89.00 ± 1.22	82.75 ± 5.81	75.75 ± 4.73
Epithelial cells	10.75 ± 1.44	8.00 ± 4.74	4.25 ± 2.39
Female			
Polymorphonuclear cells	0.250 ± 0.250	1.000 ± 0.577	16.000 ± 3.606*
Lymphocytes	0.000 ± 0.000	0.000 ± 0.000	1.333 ± 0.882*
Macrophages	84.50 ± 5.52	92.67 ± 0.88	79.00 ± 3.06
Epithelial cells	15.25 ± 5.54	6.33 ± 0.88	3.67 ± 2.33

- * Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
- ^a Units are presented as percent of total cells.

TABLE G11
Bronchoalveolar Lavage Fluid Cell Populations of Mice at the 24-Month Interim Evaluation^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Polymorphonuclear cells	0.200 ± 0.200	13.000 ± 2.345*	16.500 ± 1.803**
Lymphocytes	0.000 ± 0.000	0.375 ± 0.239	0.500 ± 0.289
Macrophages	89.10 ± 2.50	78.25 ± 1.61*	80.33 ± 0.60*
Epithelial cells	10.70 ± 2.61	8.38 ± 1.01	2.67 ± 1.59
Female			
Polymorphonuclear cells	0.000 ± 0.000	7.500 ± 1.607*	20.667 ± 5.918**
Lymphocytes	0.000 ± 0.000	0.500 ± 0.500	0.500 ± 0.500
Macrophages	86.38 ± 3.57	87.00 ± 2.08	73.67 ± 8.46
Epithelial cells	13.63 ± 3.57	5.00 ± 1.00	5.17 ± 3.03

- * Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
- ** $P \leq 0.01$
- ^a Units are presented as percent of total cells.

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Lung Burden and Lung Biochemistry of Mice

G-11

TABLE G12
Phagocytic Activity of Macrophages in Bronchoalveolar Fluid of Mice
at the 12-Month Interim Evaluation^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Phagocytic Activity	85.50 ± 1.44	56.10 ± 2.23*	16.77 ± 2.98**
Female			
Phagocytic Activity	77.07 ± 9.88	52.10 ± 9.22	17.37 ± 6.17**

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as percent cells phagocytizing sheep erythrocytes.

TABLE G13
Phagocytic Activity of Macrophages in Bronchoalveolar Fluid of Mice
at the 18-Month Interim Evaluation^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Phagocytic Activity	37.43 ± 8.55	14.10 ± 4.54	11.98 ± 2.22*
Female			
Phagocytic Activity	46.85 ± 11.08	20.03 ± 7.45	6.65 ± 0.35*

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

^a Units are presented as percent cells phagocytizing sheep erythrocytes.

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Talc, NTP TR 421

TABLE G14
Viability and Phagocytic Activity of Macrophages in Bronchoalveolar Fluid of Mice
at the 24-Month Interim Evaluation

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Viability ^a	79.20 ± 3.44	64.60 ± 4.15	83.23 ± 0.87
Phagocytic Activity ^b	37.14 ± 9.80	11.90 ± 4.64	3.56 ± 2.25**
Female			
Viability	60.50 ± 8.80	47.17 ± 2.74	59.77 ± 3.21
Phagocytic Activity	21.57 ± 6.77	13.60 ± 4.71	4.35 ± 2.65*

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as percent viable cells.

^b Units are presented as percent cells phagocytizing sheep erythrocytes.

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Lung Burden and Lung Biochemistry of Mice

G-13

TABLE G15
Measurements of Lung Collagen in Mice at the 6-Month Interim Evaluation

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Lavage Fluid Collagenous Peptides ^a	67.13 ± 9.76	24.83 ± 8.18	79.64 ± 18.03
Total Lung Collagen ^b	7.42 ± 0.48	7.51 ± 1.38	12.27 ± 4.53
Female			
Lavage Fluid Collagenous Peptides	42.92 ± 8.49	70.83 ± 9.09	51.17 ± 5.14
Total Lung Collagen	4.69 ± 0.35	5.85 ± 0.89	11.00 ± 3.88

^a Units are presented as µg/g control lung.^b Units are presented as mg/g control lung.

TABLE G16
Measurements of Lung Collagen in Mice at the 12-Month Interim Evaluation

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Lavage Fluid Collagenous Peptides ^a	74.23 ± 9.42	68.73 ± 4.11	117.62 ± 11.87 ^a
Total Lung Collagen ^b	11.94 ± 0.47	12.44 ± 0.82	13.30 ± 1.11
Female			
Lavage Fluid Collagenous Peptides	89.88 ± 12.99	73.66 ± 11.58	108.55 ± 7.56
Total Lung Collagen	11.64 ± 0.48	11.84 ± 0.45	13.78 ± 1.09

^a Significantly different (P≤0.05) from the control by Dunn's or Shirley's test^a Units are presented as µg/g control lung.^b Units are presented as mg/g control lung.

TABLE G17
Measurements of Lung Collagen in Mice at the 18-Month Interim Evaluation

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Lavage Fluid Collagenous Peptides ^a	42.54 ± 2.15	51.18 ± 5.40	70.67 ± 8.41 ^{**}
Total Lung Collagen ^b	6.60 ± 0.49	7.13 ± 0.30	9.70 ± 0.70 ^{**}
Female			
Lavage Fluid Collagenous Peptides	54.09 ± 11.27	37.68 ± 6.01	64.88 ± 6.56
Total Lung Collagen	6.16 ± 0.25	6.96 ± 0.31	7.34 ± 0.43

^{**} Significantly different (P≤0.01) from the control by Dunn's or Shirley's test^a Units are presented as µg/g control lung.^b Units are presented as mg/g control lung.

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TABLE G18
Lung Collagen Metabolism and Protein Synthesis in Mice at the 24-Month Interim Evaluation

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Lavage Fluid Collagenous Peptides ^a	54.39 ± 4.42	65.98 ± 5.01	91.92 ± 4.93**
Total Lung Collagen ^b	8.53 ± 0.71	8.55 ± 0.59	13.71 ± 2.81*
Collagen Production ^c	1.133 ± 0.274	0.779 ± 0.151	1.554 ± 0.291
Non-Collagenous Protein Synthesis ^d	18.73 ± 2.85	16.09 ± 1.15	25.64 ± 2.66
Female			
Lavage Fluid Collagenous Peptides	38.09 ± 4.38	39.26 ± 4.01	62.14 ± 9.04*
Total Lung Collagen	6.04 ± 0.27	6.41 ± 0.36	7.91 ± 0.35*
Collagen Production ^c	1.15 ± 0.33	1.65 ± 0.13	1.33 ± 0.12
Non-Collagenous Protein Synthesis ^d	17.05 ± 2.80	15.45 ± 2.26	27.46 ± 1.57

* Significantly different (P≤0.05) from the control by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as µg/g control lung.

^b Units are presented as mg/g control lung.

^c Units are presented as percent new protein.

^d Units are presented as dpm x 10⁻³/g control lung.

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Lung Burden and Lung Biochemistry of Mice

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TABLE G19

Proteinase Activity in Lavage Fluid and Lung Homogenate Supernatant Fluid of Mice at the 6-Month Interim Evaluation^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Lavage Fluid			
Acid Proteinase	1.27 ± 0.24	1.65 ± 0.47	2.05 ± 0.23
Cathepsin D	0.078 ± 0.038	0.656 ± 0.321*	0.876 ± 0.107*
Cathepsin B	1.006 ± 0.239	0.992 ± 0.716	0.954 ± 0.010
Homogenate Supernatant Fluid			
Acid Proteinase	5.83 ± 1.07	8.10 ± 0.78	7.45 ± 0.64
Cathepsin D	2.27 ± 0.46	3.30 ± 0.57	- ^b
Cathepsin B	3.56 ± 0.80	4.80 ± 0.58	-
Neutral Proteinase	0.634 ± 0.039	0.360 ± 0.043*	-
PMN Elastase Cathepsin G	0.446 ± 0.014	0.418 ± 0.357	-
Macrophage Elastase Collagenase	0.207 ± 0.058	0.340 ± 0.154	-
Female			
Lavage Fluid			
Acid Proteinase	0.762 ± 0.089	1.595 ± 0.038**	1.346 ± 0.097
Cathepsin D	0.457 ± 0.166	0.998 ± 0.016	0.628 ± 0.113
Cathepsin B	0.260 ± 0.068	0.571 ± 0.063	0.718 ± 0.094*
Homogenate Supernatant Fluid			
Acid Proteinase	4.35 ± 0.31	6.95 ± 0.61*	5.77 ± 0.61
Cathepsin D	1.78 ± 0.12	3.89 ± 1.52*	3.12 ± 0.06*
Cathepsin B	2.57 ± 0.22	3.06 ± 1.01	2.65 ± 0.56
Neutral Proteinase	0.522 ± 0.047	0.535 ± 0.039	0.848 ^c
PMN Elastase Cathepsin G	0.416 ± 0.033	0.347 ± 0.066	-
Macrophage Elastase Collagenase	0.106 ± 0.043	0.188 ± 0.058	-

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mg/hour/mg control lung.^b n=0; no data recorded^c n=1; no statistic calculated

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Tal, NTP TR 421

TABLE G20
Proteinase Activity in Lavage Fluid and Lung Homogenate Supernatant Fluid of Mice
at the 12-Month Interim Evaluation^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Lavage Fluid			
Acid Proteinase	1.65 ± 0.13	2.11 ± 0.82	3.25 ± 0.28
Cathepsin D	0.403 ± 0.163	0.970 ± 0.244	1.796 ± 0.306**
Cathepsin B	1.25 ± 0.10	1.25 ± 0.78	1.46 ± 0.05
Homogenate Supernatant Fluid			
Acid Proteinase	7.21 ± 0.50	9.35 ± 0.07*	16.50 ± 0.95**
Cathepsin D	5.32 ± 0.27	7.71 ± 0.16*	14.32 ± 1.27**
Cathepsin B	1.89 ± 0.48	1.64 ± 0.10	2.18 ± 0.39
Neutral Proteinase	0.386 ± 0.055	1.029 ± 0.416	1.088 ± 0.271*
PMN Elastase Cathepsin G	0.110 ± 0.110	0.005 ± 0.005	0.209 ± 0.148
Macrophage Elastase Collagenase	0.426 ± 0.159	1.127 ± 0.422	0.879 ± 0.162
Female			
Lavage Fluid			
Acid Proteinase	1.94 ± 0.17	1.79 ± 0.35	3.60 ± 0.33*
Cathepsin D	0.526 ± 0.263	0.463 ^b	1.525 ± 0.266*
Cathepsin B	1.50 ± 0.41	2.14 ^b	2.08 ± 0.08
Homogenate Supernatant Fluid			
Acid Proteinase	7.88 ± 0.24	10.48 ± 0.50*	16.92 ± 1.84**
Cathepsin D	6.40 ± 0.70	8.44 ± 0.51	14.76 ± 1.59**
Cathepsin B	1.55 ± 0.54	2.04 ± 0.22	2.16 ± 0.55
Neutral Proteinase	0.423 ± 0.183	0.601 ± 0.108	0.824 ± 0.057
PMN Elastase Cathepsin G	0.215 ± 0.125	0.213 ± 0.213	0.190 ± 0.124
Macrophage Elastase Collagenase	0.280 ± 0.116	0.446 ± 0.127	0.653 ± 0.158

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test

** $P \leq 0.01$

^a Units are presented as mg/hour/mg control lung.

^b n=1; no statistic calculated

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Lung Burden and Lung Biochemistry of Mice

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TABLE G21

Proteinase Activity in Lavage Fluid and Lung Homogenate Supernatant Fluid of Mice at the 18-Month Interim Evaluation^a

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Lavage Fluid			
Acid Proteinase	0.264 ± 0.044	0.428 ± 0.120	0.384 ± 0.066
Cathepsin D	0.212 ± 0.046	0.073 ± 0.013*	0.051 ± 0.035*
Cathepsin B	0.069 ± 0.037	0.355 ± 0.127*	0.342 ± 0.057*
Homogenate Supernatant Fluid			
Acid Proteinase	3.29 ± 0.58	4.76 ± 0.49	8.38 ± 0.85**
Cathepsin D	2.71 ± 0.24	4.98 ± 0.63*	8.45 ± 0.63**
Cathepsin B	0.607 ± 0.327	0.053 ± 0.053	0.403 ± 0.270
Neutral Proteinase	0.425 ± 0.079	0.548 ± 0.022	0.528 ± 0.034
PMN Elastase Cathepsin G	0.158 ± 0.066	0.242 ± 0.061	0.254 ± 0.017
Macrophage Elastase Collagenase	0.286 ± 0.093	0.306 ± 0.041	0.275 ± 0.031
Female			
Lavage Fluid			
Acid Proteinase	0.267 ± 0.103	0.561 ± 0.126	0.382 ± 0.040
Cathepsin D	0.219 ± 0.085	0.012 ± 0.012	0.062 ± 0.036
Cathepsin B	0.088 ± 0.034	0.587 ± 0.095*	0.358 ± 0.098*
Homogenate Supernatant Fluid			
Acid Proteinase	3.97 ± 0.41	5.57 ± 0.26*	9.03 ± 0.88**
Cathepsin D	3.28 ± 0.23	5.37 ± 0.16*	9.17 ± 0.75**
Cathepsin B	0.694 ± 0.284	0.232 ± 0.096	0.265 ± 0.265
Neutral Proteinase	0.381 ± 0.041	0.540 ± 0.036*	0.583 ± 0.035*
PMN Elastase Cathepsin G	0.265 ± 0.038	0.391 ± 0.038	0.268 ± 0.041
Macrophage Elastase Collagenase	0.116 ± 0.033	0.149 ± 0.054	0.315 ± 0.045*

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test

** P≤0.01

^a Units are presented as mg/hour/mg control lung.

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Talc, NTP TR 421

TABLE G22
Proteinase Activity in Lavage Fluid and Lung Homogenate Supernatant Fluid of Mice
at the 24-Month Interim Evaluation¹

	0 mg/m ³	6 mg/m ³	18 mg/m ³
Male			
Lavage Fluid			
Acid Proteinase	1.62 ± 0.14	1.92 ± 0.18	3.56 ± 0.67*
Cathepsin D	0.000 ± 0.000	0.260 ± 0.156	1.613 ± 0.632**
Cathepsin B	1.94 ± 0.19	1.72 ± 0.28	1.78 ± 0.29
Homogenate Supernatant Fluid			
Acid Proteinase	9.23 ± 1.16	13.85 ± 1.56	24.34 ± 2.66*
Cathepsin D	6.63 ± 0.96	10.82 ± 0.98*	18.75 ± 1.73**
Cathepsin B	2.60 ± 0.39	3.03 ± 0.78	5.58 ± 1.11*
Neutral Proteinase	0.417 ± 0.072	0.568 ± 0.104	0.862 ± 0.164*
PMN Elastase Cathepsin G	0.251 ± 0.034	0.382 ± 0.093	0.341 ± 0.106
Macrophage Elastase Collagenase	0.166 ± 0.063	0.186 ± 0.040	0.521 ± 0.250
Female			
Lavage Fluid			
Acid Proteinase	0.854 ± 0.077	1.012 ± 0.149	0.998 ± 0.212
Cathepsin D	0.194 ± 0.089	0.114 ± 0.114	0.402 ± 0.146
Cathepsin B	0.708 ± 0.118	1.000 ± 0.365	0.596 ± 0.305
Homogenate Supernatant Fluid			
Acid Proteinase	7.83 ± 1.11	9.76 ± 0.56	22.54 ± 1.29*
Cathepsin D	5.10 ± 0.67	8.04 ± 0.95	17.93 ± 0.55**
Cathepsin B	2.73 ± 0.47	1.71 ± 0.57	4.61 ± 1.00
Neutral Proteinase	0.454 ± 0.096	0.646 ± 0.143	0.922 ± 0.077*
PMN Elastase Cathepsin G	0.172 ± 0.063	0.341 ± 0.082	0.360 ± 0.093
Macrophage Elastase Collagenase	0.421 ± 0.293	0.314 ± 0.162	0.563 ± 0.102

* Significantly different (P ≤ 0.05) from the control group by Dunn's or Shirley's test

** P ≤ 0.01

¹ Units are presented as mg/hour/mg control lung.

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APPENDIX H

CHEMICAL CHARACTERIZATION, ANALYSIS, AND GENERATION OF CHAMBER CONCENTRATIONS

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Talc, NTP TR 421

CHEMICAL CHARACTERIZATION, ANALYSIS, AND GENERATION OF CHAMBER CONCENTRATIONS

PROCUREMENT AND CHARACTERIZATION OF TALC

Talc was obtained from Walsh and Associates (North Kansas City, MO) in two lots (lot W101882 and lot B5415). Lot W101882 was used from the beginning of the 2-year studies through January 1986. Lot B5415 was used in the 2-year studies from 27 January 1986 to the end of the studies on 31 October 1986. The talc was extensively characterized by the analytical chemistry laboratory, Midwest Research Institute (MRI; Kansas City, MO) and McCrone Associates (Norcross, GA). Reports on analyses performed in support of the talc studies are on file at the National Institute of Environmental Health Sciences.

The two lots of the chemical, a finely powdered white solid, were identified as talc by infrared spectroscopy. All spectra were consistent with those expected for the structure and with the literature spectra of talc (*Sadtler Standard Spectra*), as shown in Figure H1.

Lot W101882 was divided into three subbatches, which were analyzed separately. Each subbatch was characterized by elemental analyses, Karl Fischer water analysis, spark source mass spectrometry, and microscopic analyses. Microscopic analysis of each lot consisted of polarized light microscopy (PLM) and transmission electron microscopy (TEM). For PLM the sample was mounted in refractive index liquids and the optical parameters were determined. Dispersion staining has the advantage that small quantities of asbestos can easily be detected since the optical properties are interpreted from bright colors seen on a black background. The colors seen are the results of differences in refractive index dispersion for a liquid and a solid. TEM was performed by sonically dispersing approximately 0.1 g of talc in a solution of 0.001% methyl cellulose in particle-free water. A drop of the suspension was placed on a carbon coated 200-mesh copper grid, and 20 grid openings were examined. The detection limit was 0.1% by weight. No asbestos fibers were detected in any of the subbatches by polarized light microscopy or transmission electron microscopy.

Elemental analyses of hydrogen, magnesium, and silicon for all three subbatches of the lot were in agreement with the theoretical values for talc. The major impurities were 0.7% aluminum and 1.0% iron. Karl Fischer water analysis indicated approximately 0.2% absorbed water. Spark source mass spectrometry for the three subbatches also indicated approximately 0.1% phosphorus, 0.5% fluorine, and 0.05% calcium, while the remaining elemental impurities were less than 0.01%.

A special study was performed on this lot to determine if the sample met the American Society for Testing and Materials standard specifications for magnesium silicate. Results indicated that lot W101882 met the standard specifications.

Automated scanning electron microscopic analysis demonstrated that the talc was virtually free of silica. In the analysis a sample of talc is suspended in methylcellulose. Under computer control the particles are located, and maximum, minimum, and average diameters are determined; then a chemical analysis is performed. Of the 1,466 particles that were examined, 1 was identified as silica, 1,241 were talc, 136 were of tremolite type composition, 77 were mixed silicates, 1 was possibly zircon, and 10 were not identified. The single silica particle had an average diameter of 3.9 μm .

Lot B5415 was characterized by elemental analyses, Karl Fischer water analysis, spark source mass spectrometry, and microscopic analyses using the same methods described for lot W101882. Elemental analyses values were similar to results obtained for lot W101882. The major impurities present were 0.1% calcium, 0.5% aluminum, and 1% iron. Karl Fischer water analysis indicated 1.2% absorbed water. Spark source mass spectrometry also indicated 0.04% phosphorus, >0.5% aluminum, 0.03% sodium, 0.35% fluorine, and all other impurities were less than 0.03%. Microscopic analyses using PLM and TEM detected no asbestos fibers.

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Chemical Characterization and Dose Formulation

H-3

Comparative purity analyses of the two lots used in these studies were conducted due to problems with the generation of inhalation concentrations. Four samples of talc were used, two samples each from lots W101882 and B5415. Samples A and B were from lot W101882, sample C was from lot B5415, and sample D was a frozen reference from lot B5415 that had been stored at MRI.

Analyses performed included elemental analyses, microscopic analyses (PLM, TEM, determination of particle size distribution, and aspect ratios), X-ray diffraction, and thermogravimetric analysis (TGA). PLM and TEM analyses were performed on samples C and D. Analysis by PLM followed the procedures described earlier; TEM followed the same procedure described earlier except the talc was sonically dispersed in a solution of 90% (v/v) isopropanol in particle-free water. The determinations of particle size distribution and aspect ratios were performed on all four samples. Using TEM for both analyses, selected area diffraction (SAD) patterns were used to confirm that the particles being measured were talc. The particle size was taken as the average of two diameters 90° to each other and aspect ratios were taken as the ratio of the two diameters. Thermogravimetric analysis (TGA) was performed on samples A, B, and C on a DuPont 910 differential scanning calorimeter (DSC) with calcium oxalate monohydrate used as a calibrating standard, at an initial temperature of 50° C with a programmed maximum temperature of 1,100° C, at a rate of 20° C per minute.

Elemental analyses for hydrogen, magnesium, and silicon for all four samples were in agreement with theoretical values. Polarized light microscopy (PLM) and transmission electron microscopy (TEM) detected no asbestos fibers in any of the samples. The results for particle size distribution and aspect ratios indicated that there were only minor differences in particle size between the samples and more than 75% of the particles were in the 1.0 to 3.0 μm range. More than 90% of the talc particles had aspect ratios between 1 and 1.4, and less than 1% had ratios greater than 3:1. X-ray diffraction confirmed that all four samples were primarily talc with small quantities of chlorite and dolomite. Thermogravimetric analysis indicated that samples A, B, and C were similar. A main peak at 912° C in all three samples caused by the loss of chemically combined water was equal to a loss of 4.7% by weight. A minor peak at 590° C in all three samples may represent the loss of CO₂ from dolomite and amounted to a loss of 0.7% by weight which is equivalent to 1.5% dolomite.

Size Distribution Analysis of Talc Samples
(% of Total Particles Counted)

Size Range (μm)	Talc A	Talc B	Talc C	Talc D
0.5-1.0	5.88	2.97	12.50	1.94
1.0-1.5	15.69	9.90	19.23	11.65
1.5-2.0	26.47	26.73	24.04	26.21
2.0-2.5	20.59	17.82	21.15	23.30
2.5-3.0	11.76	18.81	10.58	8.74
3.0-3.5	5.88	12.87	4.81	7.77
3.5-4.0	3.92	5.94	2.88	5.83
4.0-4.5	2.94	1.98	1.92	4.85
4.5-5.0	2.94	0.99	0.96	3.88
5.0-5.5	1.96	0.99	0.96	2.91
5.5-6.0	1.96	0.99	0.96	1.94
6.0-6.5	-	-	-	0.97

The moisture content of the bulk chemical was reanalyzed every 4 months at the study laboratory by determining the weight loss following heating at 120°C for 16 hours. The results indicated that the moisture content of the talc was similar between the two lots and did not change during the 2-year studies. Bulk chemical stability studies were not performed on talc because the physical and chemical properties of talc indicate that it should be stable over a wide range of temperatures. The compound was stored in tightly sealed plastic bags at 25° C.

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H-4

Talc, NTP TR 421

GENERATION AND MONITORING OF CHAMBER CONCENTRATIONS

Aerosol Generation System: Talc aerosol was generated from one 4-inch, fluid bed generator (FBG). Figure H2 shows the schematic of the FBG with the gravity feed and collecting pan collection systems. The FBG bed contained type 316 stainless steel powder (Hoeganaes Corporation, Riverton, NJ), consisting of irregularly shaped particles 125 to 180 μm in diameter. The stainless steel powder was cleaned prior to use. The cleaning system used a 4-inch FBG with dry, filtered air flowing through at a flow rate of 80 CFM. The high flow rate through the bed removed the finest stainless steel particles. The cleaning system was run for 24 hours to ensure that all the "fines" were removed.

Following cleaning of the bed material, talc was mixed with the stainless steel powder at approximately 1 to 2.5 g of talc per 500 g bed material. The concentration of talc in the bed material was one method used to adjust exposure concentrations in the chamber. During the time period of November 1985 to January 1986, when difficulty in maintaining target concentrations was experienced, higher loadings were used in an effort to maintain target concentrations.

For generation of the talc aerosol, fluidization of the bed material mixed with talc occurred when compressed air (≈ 200 Lpm) was injected into the bed through a porous metal distribution plate which supports the bed. The motion of the bed released the much smaller talc particles into the air; the larger, heavier stainless steel particles were retained in the bed. A Kr-85 discharger was placed above the bed to reduce the particle charges. The aerosolized talc particles were mixed with diluting air (≈ 200 Lpm) to achieve the desired concentrations and were then delivered to the exposure chambers (Figures H3 and H4). As the talc powder was removed from the bed, the bed material was continually drained from the FBG through an overflow port located at the side of the generator. As spent bed material was drained from the generator, fresh talc-containing bed material was constantly added into the generator from a hopper located above the generator.

Stainless steel multitiered whole-body exposure chambers (H2000, Lab Products, Inc.) were used to expose the rats in this study while the smaller H1000 chambers were used for the mice. Flow rates through the chambers were 12 ± 2 CFM. To reduce the spatial variation of aerosol concentration and to increase the uniformity of mixing, the aerosol was diluted using a dilutor prior to its introduction into the chamber. Also, animal cages were rotated once per week to reduce the variation of concentrations of talc aerosols that the rodents were exposed to during the 2-year studies.

Aerosol Concentration Monitoring: Aerosol concentrations in each exposure chamber were monitored by taking filter samples for three, 2-hour periods during each 6-hour exposure day. The background concentration of total suspended particles in each control chamber was monitored each exposure day by taking one 6-hour filter sample. Overnight filter samples for total suspended particles were taken from the 18 mg/m^3 chambers once per month. All filter samples were taken at a flow rate of 3 L/minute. Each filter was weighed before and after the sample was taken, and the aerosol mass concentrations were calculated by dividing the mass increment (mg) by the volume sampled (m^3); the means and standard deviations for each chamber were calculated for each exposure day. Weekly mean exposure concentrations for the 2-year studies are presented in Figures H5 through H8. The concentrations during non-exposure hours in the 18 mg/m^3 chambers ranged from 0.02 to 1.1 mg/m^3 .

A RAM-S continuous aerosol monitor was used to monitor the stability of the aerosol concentrations and to determine the need to adjust the aerosol generation system during exposures. The RAM-S was used to monitor each chamber for at least 5 minutes at the beginning, middle, and end of the filter sampling period. A 2 L/minute flow rate through the RAM-S was achieved using an internal pump in the device. Both RAM-S and filter samples were taken at one point of the chambers above the animal cage. A Y-shaped probe was used, allowing simultaneous filter sampling and RAM-S aerosol mass monitor operation. The overall temporal variation in chamber concentrations in the 2-year studies were 33% and 27% relative standard deviation (RSD) for the mouse 6 and 18 mg/m^3 chambers. The variations were 31% and 36% RSD for the rat 6 and 18 mg/m^3 chambers. At least a portion of this variability may be ascribed to the period when talc generation problems were encountered (November 1985 through February 1986). In addition, a portion of the variability for the 18 mg/m^3 rat chamber

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may be ascribed to the time when higher concentrations were being generated (September through November, 1984).

During the period of November 5, 1985 through January 27, 1986 difficulties were experienced maintaining the required exposure levels of talc for the lifetime and 2-year exposure studies. Concentrations of aerosolized talc were significantly below target. Attempts were made to increase the flow of talc into the generator and raise the concentration; however, the talc-laden stainless steel bed material fed into the generator less freely than it had prior to November, 1985. There were no observable chemical changes in either the talc or the stainless steel bed material and no malfunctions in the generation system which could be pinpointed as the underlying cause for the poor flow characteristics of the bed material. On January 27, 1986, the generator was restarted with a new batch of talc. After a stabilization period of three weeks, the flow properties of the bed material showed significant improvement.

It was also observed during February, 1986 that when the ratio of talc to bed material was increased above 1.6 g talc per 500 g bed material, the bed began to show the poor flow properties characteristic of the previous batch of talc. When the bed loading was reduced below 1.6 g talc per 500 g bed material, the flow properties stabilized. This indicated that the bed has a maximum loading limit which must not be exceeded. By March 1986, the generator had stabilized and chamber target concentrations were achieved. The exact cause of these generation problems was never resolved.

In November, 1984 it was noticed that the RAM-S monitor indicated an off-scale reading (>10 V which is equivalent to 20 mg/m^3) for the 18 mg/m^3 rat chamber. Reasonable agreement was seen between RAM-S readings and filter samples in the other chambers. Investigations of this discrepancy indicated that the airflow through the critical orifice controlling flow through the filter was reduced. Evaluation of the previously collected pressure drop associated with this orifice and one having nearly identical nominal flow revealed that the flow to the sampling filter of the high level rat chamber dropped significantly on September 24, 1984. These data suggest that the sampling orifice had become partially clogged. In order to obtain a correction factor to recalculate the chamber concentration data, the filter pressure drop and exposure chamber pressure drop data were retrieved and used to determine the actual pressure drop across the sampling filter for the time period of September 24 through November 14, 1984. A group of 18 filters from different lots of the type used to sample the talc exposure chambers were tested to determine the pressure drop across them as a function of the flow through the filter. These data indicated that values for flow could be calculated from the pressure drop data. The relationship between pressure drop and filter flow rate was used to recalculate the sampling filter flow for each day. When the chamber sampling orifice flow rate was taken into account, the best estimate of the correction factor is 2.06. This factor has been used to multiply the originally recorded chamber concentrations for those dates. The corrected values are reported.

Aerosol size distribution was determined once a month for each chamber using a cascade impactor operated at a flow rate of 15 L/minute. Stainless steel disks coated with apiezon grease were used as impactor substrates and the amount of talc collected on each stage was determined by the difference in stage weight before and after the sample was taken. The mass median aerodynamic diameter and the geometric standard deviation were calculated from the mass data, effective cutoff diameter of each stage, and impactor flow rate. The results are presented in Tables H1 and H2.

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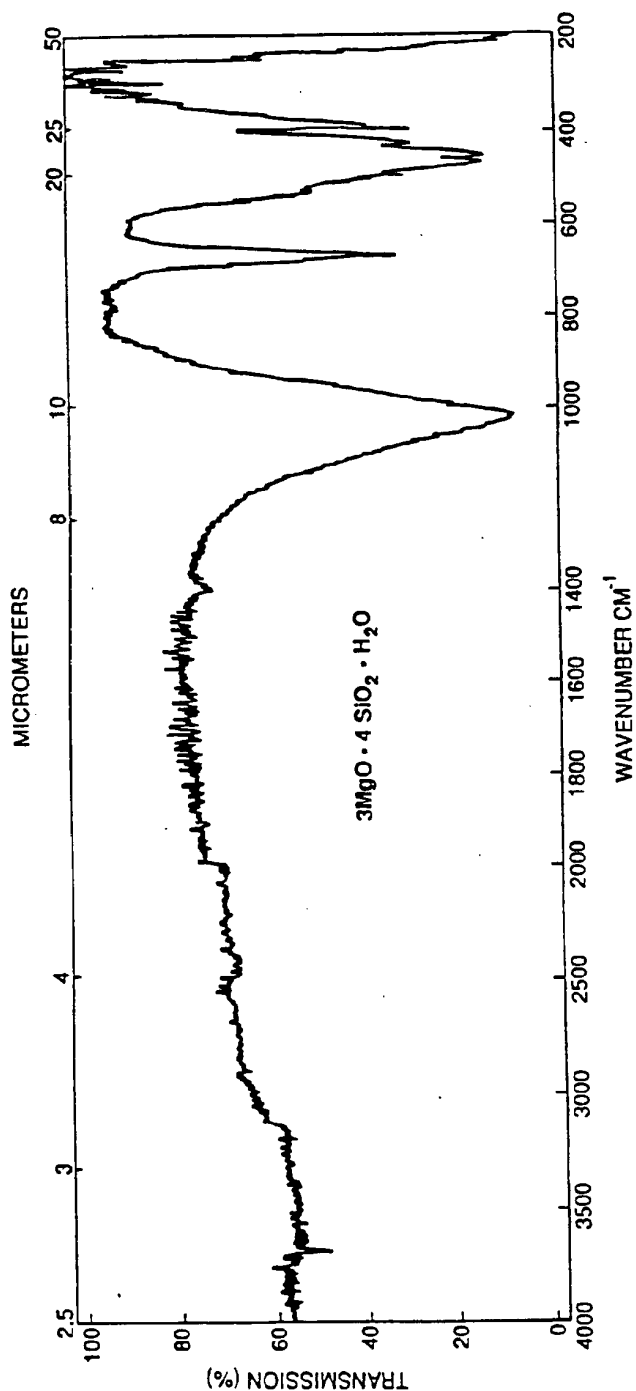


FIGURE H1
Infrared Absorption Spectrum of Talc

ABSCISSA EXPANSION 1		ORDINATE EXPANSION 1		SCAN TIME 24 min		REP. SCAN	
SUPPRESSION		%T 0-100 ABS		RESPONSE 1		TIME DRIVE	
SAMPLE: Talc Lot W101882 Batch 02 Subbatch A		REMARKS Trimmer comb in reference beam		SLIT PROGRAM 6		OPERATOR A. Clark	
				SOLVENT		CELL PATH	
				CONCENTRATION 1% in KBr		REFERENCE 154N	
						DATE 11/9/92	

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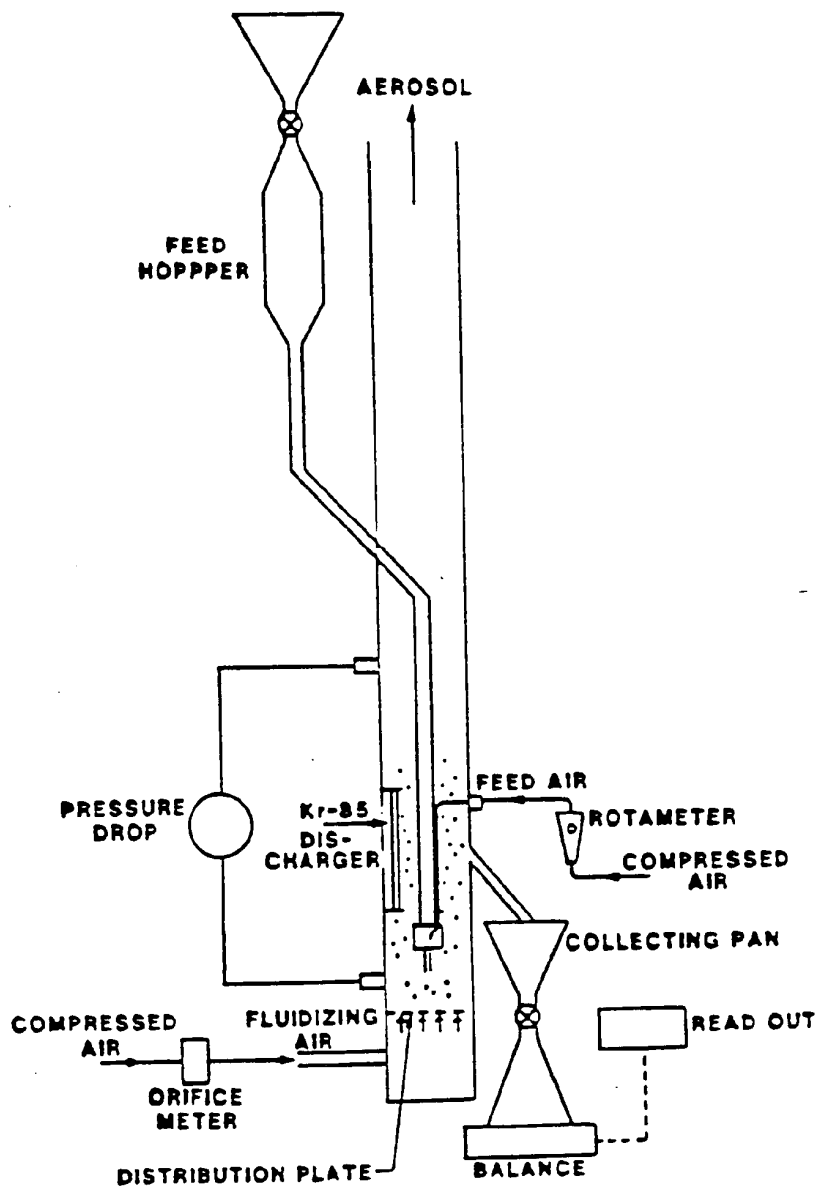


FIGURE H2
Fluid Bed Generator

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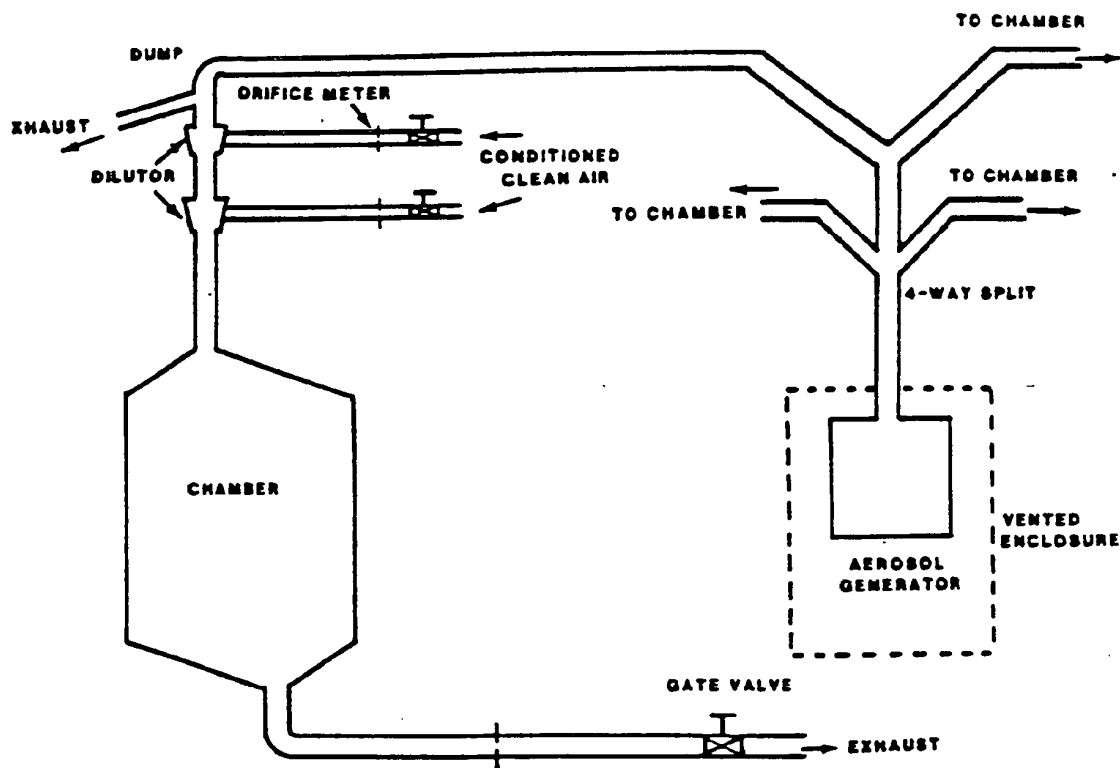


FIGURE H3
Aerosol Dilution/Delivery System

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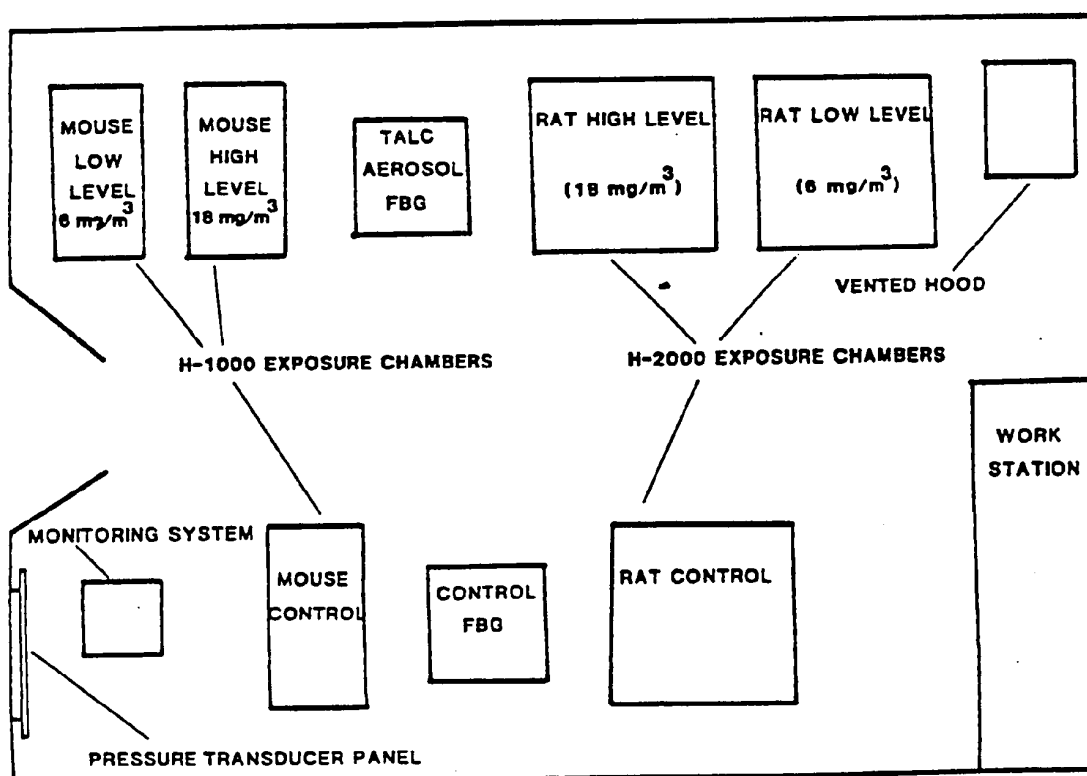


FIGURE H4
Talc Chronic Exposure System

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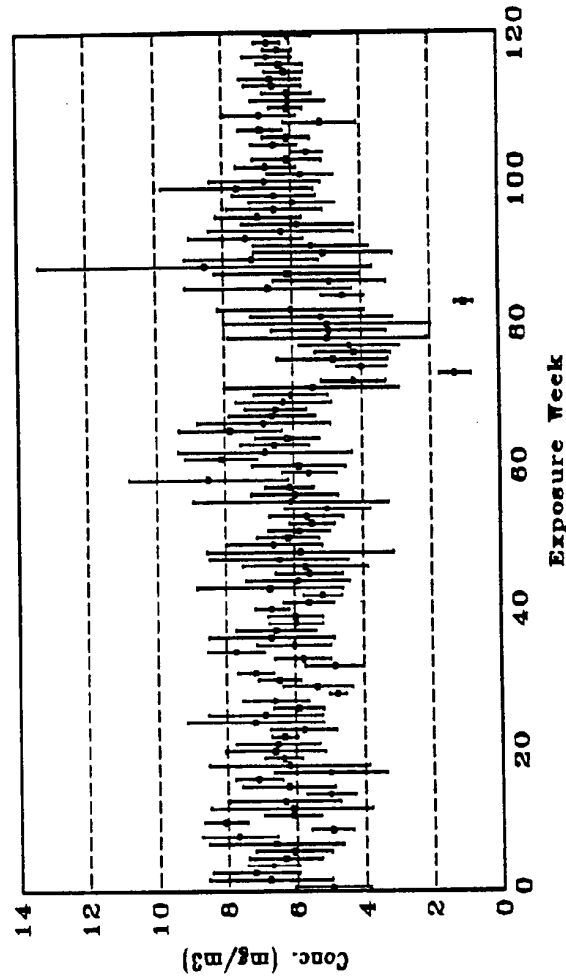


FIGURE H5
Talc Aerosol Filter Concentrations in the 6 mg/m³ Rat Chamber

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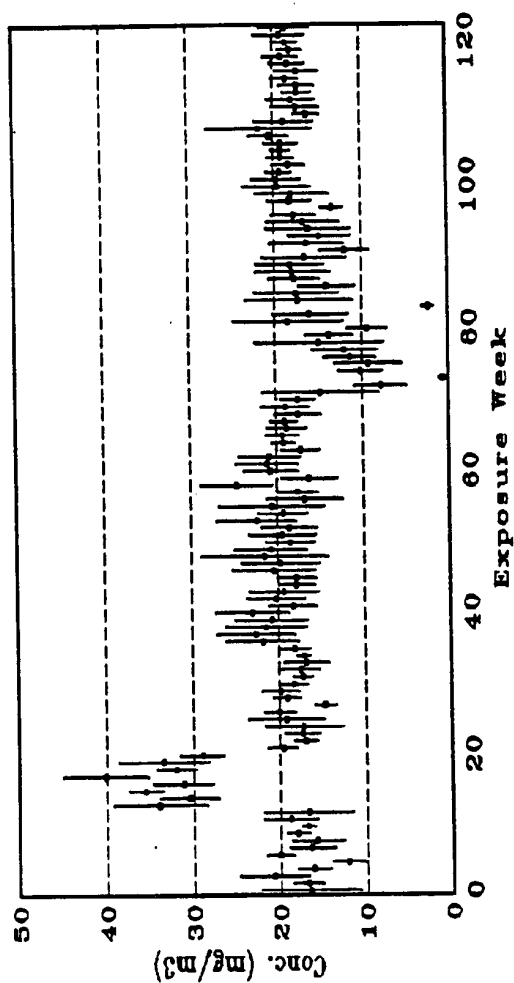


FIGURE H6
Talc Aerosol Filter Concentrations in the 18 mg/m³ Rat Chamber

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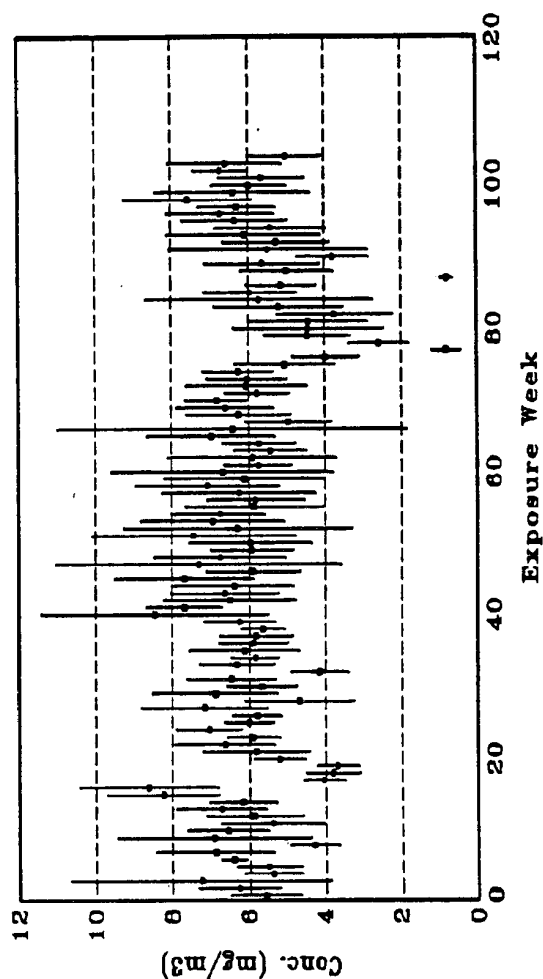


FIGURE H7
Talc Aerosol Filter Concentrations in the 6 mg/m³ Mice Chamber

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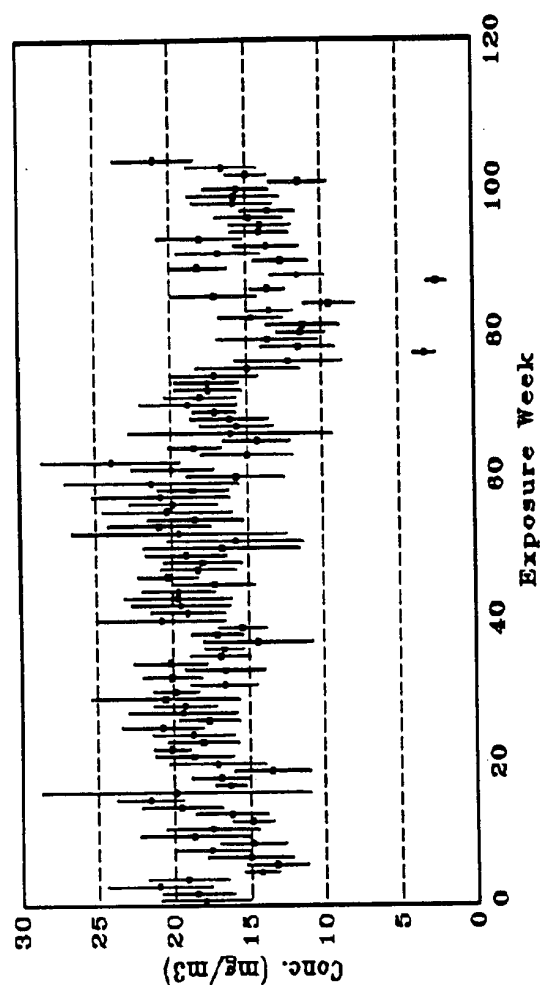


FIGURE H8
Talc Aerosol Filter Concentrations in the 18 mg/m³ Mice Chamber

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TABLE H1
Summary of Aerosol Size Measurements for the 6 and 18 mg/m³ Rat Chambers

6 mg/m ³			18 mg/m ³		
Date	Mass Median Aerodynamic Diameter (μm)	Geometric Standard Deviation	Date	Mass Median Aerodynamic Diameter (μm)	Geometric Standard Deviation
9 July 1984	2.3	2.6	25 June 1984	3.6	2.0
6 August 1984	2.6	1.7	1 August 1984	3.0	1.8
4 September	2.8	1.8	27 August 1984	3.2	1.9
3 October 1984	2.6	1.8	26 September 1984	2.9	1.8
31 October 1984	2.9	1.8	24 October 1984	3.2	1.9
27 November 1984	2.5	1.8	20 November 1984	3.0	1.9
4 January 1985	2.6	1.8	24 December 1984	2.8	1.8
25 January 1985	2.5	1.7	14 January 1985	2.9	1.8
25 February 1985	2.6	1.8	19 February 1985	2.8	1.8
19 March 1985	2.8	1.8	15 March 1985	3.1	2.0
22 April 1985	2.9	1.7	12 April 1985	3.1	1.8
13 June 1985	3.0	1.9	8 May 1985	2.9	1.9
9 July 1985	2.8	1.8	10 June 1985	3.0	1.9
9 August 1985	2.7	1.9	5 July 1985	3.5	1.8
3 September 1985	2.7	1.5	1 August 1985	3.1	1.9
30 September 1985	2.3	1.3	26 August 1985	2.9	1.9
28 October 1985	2.6	1.4	23 September 1985	2.6	1.6
2 December 1985	3.1	1.7	21 October 1985	2.7	1.5
18 December 1985	3.0	1.7	25 November 1985	4.0	2.1
3 January 1986	1.8	2.8	17 December 1985	3.3	1.9
8 January 1986	3.6	1.9	30 December 1985	3.7	1.8
13 January 1986	3.1	1.8	3 January 1986	4.0	2.2
24 February 1986	2.9	2.2	8 January 1986	3.8	1.9
24 March 1986	3.4	1.9	18 February 1986	3.2	2.1
22 April 1986	3.2	2.3	17 March 1986	3.6	1.9
23 May 1986	2.4	1.9	14 April 1986	4.0	2.0
23 May 1986	2.9	1.9	19 May 1986	3.2	1.8
27 May 1986	2.3	1.9	2 June 1986	3.2	2.1
16 June 1986	2.7	2.7	17 June 1986	3.3	1.9
30 June 1986	2.2	2.4	15 July 1986	3.4	2.0
28 July 1986	2.5	2.3	11 August 1986	3.1	1.9
25 August 1986	2.1	2.5	9 September 1986	2.9	1.9
22 September 1986	2.5	2.0	6 October 1986	2.7	2.3
20 October 1986	2.7	2.3			
Mean ± standard deviation	2.7 ± 0.4	1.9 ± 0.4		3.2 ± 0.4	1.9 ± 0.2

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TABLE H2

Summary of Aerosol Size Measurements for the 6 and 18 mg/m³ Mouse Chambers

6 mg/m ³			18 mg/m ³		
Date	Mass Median Aerodynamic Diameter (μm)	Geometric Standard Deviation	Date	Mass Median Aerodynamic Diameter (μm)	Geometric Standard Deviation
18 June 1984	3.9	1.8	25 June 1984	3.6	2.0
16 July 1984	3.4	1.9	23 July 1984	3.7	1.9
14 August 1984	3.5	1.8	20 August 1984	3.5	1.8
18 September 1984	3.3	1.8	10 September 1984	3.9	2.0
10 October 1984	3.1	1.9	17 October 1984	3.8	1.9
7 November 1984	3.3	1.8	19 November 1984	3.5	1.7
4 December 1984	3.0	1.8	12 December 1984	3.3	1.9
7 January 1985	3.4	1.6	7 January 1985	3.4	1.8
4 February 1985	3.2	1.8	8 February 1985	3.6	1.9
1 March 1985	2.9	1.9	7 March 1985	3.6	1.9
29 March 1985	3.1	1.8	5 April 1985	3.5	1.9
23 April 1985	3.6	1.8	2 May 1985	3.6	1.8
22 May 1985	3.1	2.0	29 May 1985	3.5	2.2
21 June 1985	3.3	1.8	26 June 1985	3.7	2.0
23 July 1985	3.4	1.8	29 July 1985	3.5	1.9
15 August 1985	3.5	1.8	20 August 1985	3.8	1.9
9 September 1985	2.6	1.3	16 September 1985	3.3	1.8
7 October 1985	2.7	1.5	14 October 1985	2.8	1.7
4 November 1985	2.5	1.5	12 November 1985	4.1	2.1
9 December 1985	3.4	1.6	16 December 1985	3.8	2.0
19 December 1985	3.6	2.0	3 January 1986	3.6	1.9
3 January 1986	3.9	2.0	8 January 1986	5.0	2.0
8 January 1986	4.0	2.1	10 February 1986	3.3	2.4
20 January 1986	3.7	1.8	13 March 1986	3.1	2.5
3 March 1986	3.0	2.1	7 April 1986	3.4	2.0
31 March 1986	2.9	2.1	5 May 1986	3.3	2.2
28 April 1986	3.2	4.7			
Mean ± standard deviation	3.3 ± 0.4	1.9 ± 0.6		3.6 ± 0.4	2.0 ± 0.2

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**APPENDIX I
INGREDIENTS, NUTRIENT COMPOSITION,
AND CONTAMINANT LEVELS
IN NIH-07 RAT AND MOUSE RATION**

TABLE I1	Ingredients of NIH-07 Rat and Mouse Ration	I-2
TABLE I2	Vitamins and Minerals in NIH-07 Rat and Mouse Ration	I-2
TABLE I3	Nutrient Composition of NIH-07 Rat and Mouse Ration	I-3
TABLE I4	Contaminant Levels in NIH-07 Rat and Mouse Ration	I-4

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TABLE I1
Ingredients of NIH-07 Rat and Mouse Ration^a

Ingredients ^b	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Dried brewer's yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

^a NCI, 1976; NIH, 1978

^b Ingredients were ground to pass through a U.S. Standard Screen No. 16 before being mixed.

TABLE I2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration^a

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D ₃	4,600,000 IU	D-activated animal sterol
K ₃	2.8 g	Menadione
d-α-Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	d-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B ₁₂	4,000 µg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	d-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

^a Per ton (2,000 lb) of finished product

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Feed Analyses

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TABLE I3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrient	Mean \pm Standard Deviation	Range	Number of Samples
Protein (% by weight)	22.22 \pm 0.72	21.1-23.5	13
Crude fat (% by weight)	5.59 \pm 0.55	4.7-6.4	13
Crude fiber (% by weight)	3.36 \pm 0.30	2.7-3.8	13
Ash (% by weight)	6.55 \pm 0.23	6.1-7.0	13
Amino Acids (% of total diet)			
Arginine	1.308 \pm 0.606	1.210-1.390	8
Cystine	0.306 \pm 0.084	0.181-0.400	8
Glycine	1.150 \pm 0.047	1.060-1.210	8
Histidine	0.576 \pm 0.024	0.531-0.607	8
Isoleucine	0.917 \pm 0.029	0.881-0.944	8
Leucine	1.946 \pm 0.055	1.850-2.040	8
Lysine	1.270 \pm 0.058	1.200-1.370	8
Methionine	0.448 \pm 0.128	0.306-0.699	8
Phenylalanine	0.987 \pm 0.140	0.665-1.110	8
Threonine	0.877 \pm 0.042	0.824-0.940	8
Tryptophane	0.236 \pm 0.176	0.107-0.671	8
Tyrosine	0.676 \pm 0.105	0.564-0.794	8
Valine	1.103 \pm 0.040	1.050-1.170	8
Essential Fatty Acids (% of total diet)			
Linoleic	2.393 \pm 0.258	1.830-2.570	7
Linolenic	0.280 \pm 0.040	0.210-0.320	7
Vitamins			
Vitamin A (IU/kg)	9,846 \pm 2,839	5,600-15,000	13
Vitamin D (IU/kg)	4,450 \pm 1,382	3,000-6,300	4
α -Tocopherol (ppm)	37.95 \pm 9.41	22.5-48.9	8
Thiamine (ppm)	20.77 \pm 2.01	17.0-23.0	13
Riboflavin (ppm)	7.92 \pm 0.87	6.10-9.00	8
Niacin (ppm)	103.4 \pm 26.59	65.0-150.0	8
Pantothenic acid (ppm)	29.54 \pm 3.60	23.0-34.0	8
Pyridoxine (ppm)	9.55 \pm 3.48	5.60-14.0	8
Folic acid (ppm)	2.25 \pm 0.73	1.80-3.70	8
Biotin (ppm)	0.254 \pm 0.042	0.19-0.32	8
Vitamin B ₁₂ (ppb)	38.45 \pm 22.01	10.6-65.0	8
Choline (ppm)	3,089 \pm 328.69	2,400-3,430	8
Minerals			
Calcium (%)	1.17 \pm 0.09	1.06-1.41	13
Phosphorus (%)	0.92 \pm 0.03	0.87-0.99	13
Potassium (%)	0.883 \pm 0.078	0.772-0.971	6
Chloride (%)	0.526 \pm 0.092	0.380-0.635	8
Sodium (%)	0.313 \pm 0.390	0.258-0.371	8
Magnesium (%)	0.168 \pm 0.010	0.151-0.181	8
Sulfur (%)	0.280 \pm 0.064	0.208-0.420	8
Iron (ppm)	360.5 \pm 100	255.0-523.0	8
Manganese (ppm)	92.0 \pm 6.01	81.70-99.40	8
Zinc (ppm)	54.72 \pm 5.67	46.10-64.50	8
Copper (ppm)	11.06 \pm 2.50	8.090-15.39	8
Iodine (ppm)	3.37 \pm 0.92	1.52-4.13	6
Chromium (ppm)	1.79 \pm 0.36	1.04-2.09	8
Cobalt (ppm)	0.681 \pm 0.14	0.490-0.780	4

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TABLE I4
Contaminant Levels in NIH-07 Rat and Mouse Ration

	Mean \pm Standard Deviation ^a	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.72 \pm 0.19	0.33–0.94	13
Cadmium (ppm)	<0.1		13
Lead (ppm)	0.57 \pm 0.31	0.14–1.32	13
Mercury (ppm)	<0.05		13
Selenium (ppm)	0.35 \pm 0.08	0.21–0.44	13
Aflatoxins (ppb)	<5.0		13
Nitrate nitrogen (ppm) ^b	12.56 \pm 4.47	2.80–18.0	13
Nitrite nitrogen (ppm) ^b	0.14 \pm 0.11	<0.10–0.50	13
BHA (ppm) ^c	2.54 \pm 1.05	<2.00–5.00	13
BHT (ppm) ^c	2.39 \pm 1.33	<1.00–4.00	13
Aerobic plate count (CFU/g) ^d	39,523 \pm 39,878	3,400–130,000	13
Coliform (MPN/g) ^e	3.72 \pm 1.79	<3.00–9.00	11
Coliform (MPN/g) ^f	9.46 \pm 14.11	<3.00–43.0	13
<i>E. coli</i> (MPN/g) ^g	3.08 \pm 0.28	<3.0–4.00	13
Total nitrosamines (ppb) ^h	6.99 \pm 4.13	1.80–16.00	13
N-Nitrosodimethylamine (ppb) ^h	5.67 \pm 3.79	0.80–15.00	13
N-Nitrosopyrrolidine (ppb) ^h	1.32 \pm 0.73	1.00–3.40	13
Pesticides (ppm)			
α -BHC ⁱ	<0.01		13
β -BHC	<0.02		13
γ -BHC	<0.01		13
δ -BHC	<0.01		13
Heptachlor	<0.01		13
Aldrin	<0.01		13
Heptachlor epoxide	<0.01		13
DDE	<0.01		13
DDD	<0.01		13
DDT	<0.01		13
HCB	<0.01		13
Mirex	<0.01		13
Methoxychlor	<0.05		13
Dieldrin	<0.01		13
Endrin	<0.01		13
Telodrin	<0.01		13
Chlordane	<0.05		13
Toxaphene	<0.1		13
Estimated PCBs	<0.2		13
Ronnel	<0.01		13
Ethion	<0.02		13
Trithion	<0.05		13
Diazinon	<0.1		13
Methyl parathion	<0.02		13
Ethyl parathion	<0.02		13
Malathion ^j	0.09 \pm 0.07	0.05–0.28	13
Endosulfan I	<0.01		13
Endosulfan II	<0.01		13
Endosulfan sulfate	<0.03		13

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TABLE I4
Contaminant Levels in NIH-07 Rat and Mouse Ration (continued)

-
- ^a For values less than the limit of detection, the detection limit is given for the mean.
 - ^b Sources of contamination: alfalfa, grains, and fish meal
 - ^c Sources of contamination: soy oil and fish meal
 - ^d CFU = colony forming unit
 - ^e MPN = most probable number
 - ^f Includes two high values of 39 and 43 MPN/g obtained from lots milled 15 March 1984 and 9 May 1984, respectively.
 - ^g One lot milled 17 October 1984 contained 4.00 MPN/g; all other lots contained 3.00 MPN/g
 - ^h All values were corrected for percent recovery.
 - ⁱ BHC = hexachlorocyclohexane or benzene hexachloride.
 - ^j Seven lots contained more than 0.05 ppm.

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APPENDIX J SENTINEL ANIMAL PROGRAM

METHODS	J-2
TABLE J1 Murine Virus Antibody Determinations for Rats and Mice in the Lifetime and 2-Year Inhalation Studies of Talc	J-4

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SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Rats

Prior to the beginning of the lifetime study, 5 F344/N rats of each sex were sacrificed and serum samples were taken for serological evaluation by Microbiological Associates (Bethesda, MD). Serum samples were also taken from selected rats for serology testing at each of the interim evaluations: 3 male and 3 female rats at 6 months; 8 male and 9 female rats at 12 and 18 months; 11 male and 17 female rats at 24 months; and 15 male and 15 female rats at the terminal sacrifice (male, 113 weeks; female, 122 weeks). Blood collected from each animal was allowed to clot and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates (Bethesda, MD) for determination of antibody titers. The following tests were performed:

Method of Analysis

Time of Analysis

ELISA

RCV/SDA (rat corona virus/sialodacryoadenitis virus)	Study initiation, 6, 12, 18, 24 months, study termination
PVM (pneumonia virus of mice)	6, 12, 18, 24 months, study termination
Sendai	6, 12, 18, 24 months, study termination
<i>Mycoplasma arthritidis</i>	12, 18, 24 months, study termination
<i>Mycoplasma pulmonis</i>	12, 18, 24 months, study termination
CARB (cilia-associated respiratory bacillus)	Study termination (males only)

Hemagglutination Inhibition

H-1 (Toolan's H-1 virus)	Study initiation, 6, 12, 18, 24 months, study termination
KRV (Kilham rat virus)	Study initiation, 6, 12, 18, 24, study termination
PVM	Study initiation
Sendai	Study initiation

Immunofluorescence Assay

KRV	24 months (males only)
RCV (rat corona virus)	24 months (males only)
RCV/SDA	28 months (males only)

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Sentinel Animal Program

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Mice

Prior to the beginning of the 2-year study, 5 B6C3F₁ mice of each sex were sacrificed and serum samples were taken for serological evaluation by Microbiological Associates (Bethesda, MD). Serum samples for serology testing were also taken from control males and females at each of the interim evaluations (4 males and 4 females at 6 months; 12 males and 12 females at 12 months) and at the terminal sacrifice (15 males and 15 females). (Samples were inadvertently omitted for mice evaluated after 18 months of exposure on 4-5 December, 1985.) Blood collected from each animal was allowed to clot and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates (Bethesda, MD) for determination of antibody titers. The following tests were performed:

Method of Analysis

Time of Analysis

Complement Fixation

LCM (lymphocytic choriomeningitis virus)
Mouse adenoma virus

Study initiation, 6, 12, 24 months
Study initiation

ELISA

Ectromelia virus
GDVII (mouse encephalomyelitis virus)
MHV (mouse hepatitis virus)
PVM
Sendai
Reo 3
Mouse adenoma virus
M. arthritidis
M. pulmonis

6, 12, 24 months
Study initiation, 6, 12, 24 months
Study initiation, 6, 12, 24 months
6, 12, 24 months
6, 12, 24 months
6, 12, 24 months
6, 12, 24 months
6, 12, 24 months
6, 12, 24 months

Hemagglutination Inhibition

Ectromelia virus
K (papovirus)
MVM (minute virus mice)
PVM
Polyoma virus
Reovirus 3
Sendai

Study initiation
12, 24 months
Study initiation, 6, 12, 24 months
Study initiation
Study initiation, 6, 12, 24 months
Study initiation
Study initiation

Immunofluorescence Assay

EDIM (Epizootic diarrhea of infant mice)
Reovirus 3

6, 12, 24 months
24 months

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J-4

Talc, NTP TR 421

TABLE J1
Murine Virus Antibody Determinations for Rats and Mice in the Lifetime and 2-Year Inhalation Studies of Talc

Interval (months)	Incidence of Antibody in Sentinel Animals	Positive Serologic Reaction for
Rats		
6 months	0/6	-
12 months	0/17	-
18 months	0/17	-
24 months (males)	1/11 9/11 6/11	KRV Sendai RCV
(females)	13/17 13/17	Sendai RCV/SDA
28 months	15/15 3/15	Sendai RCV/SDA
30 months	15/15 1/15	Sendai RCV/SDA
Mice		
6 months	0/8	-
12 months	0/24	MHV
24 months	2/30 7/30 21/30	Reovirus 3 <i>M. arthritidis</i> EDIM

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K-1

APPENDIX K 4-WEEK INHALATION STUDIES IN RATS AND MICE

EXPERIMENTAL PROTOCOL	K-2
TABLE K1 Experimental Design and Materials and Methods in the 4-Week Inhalation Studies of Talc	K-3
RESULTS	K-5

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K-2

Talc, NTP TR 421

EXPERIMENTAL PROTOCOL

Procurement and Characterization of Talc

Talc was obtained from Walsh and Associates (North Kansas City, MO) in one lot (lot number W101882). Identity and purity analyses were performed by the analytical chemistry laboratory, Midwest Research Institute (MRI; Kansas City, MO).

The study chemical, a finely powdered white solid, was identified as talc by infrared spectroscopy, elemental analysis, and microscopic analyses. The moisture content of the bulk chemical was analyzed and was determined to be stable throughout the studies. Bulk chemical studies were not conducted due to the physical and chemical properties of talc. The compound was stored in sealed Nalgene containers.

Generation and Monitoring of Chamber Concentrations

Talc aerosols were generated in a fluidized bed generator by injecting filtered air into the bed. Samples were collected continuously during the 6-hour exposure day on glass fiber filters. Only one sampling port position was used each day to collect the samples from each chamber. Once a week, samples were collected on Zefluor filters so that the magnesium content of aerosolized talc could be determined and be compared to the magnesium content of bulk talc. Cascade impactor samples were taken 3 to 6 times a week to determine aerosol particle size. The amount of talc collected on the filters and impactor stages was quantitated gravimetrically. The extent of carry over of the stainless steel material from the FBG was quantitated by measuring the amount of acid soluble nickel and chromium in filter samples taken from the exposure atmosphere twice during the study.

Study Design

Groups of 10 male and 10 female F344/N rats and B6C3F₁ mice were exposed by inhalation to talc at target concentrations of 0 (chamber controls), 2, 6, and 18 mg/m³. Rats and mice were exposed for 6 hours daily, 5 days a week, for 20 days.

Source and Specification of Animals

Male and female F344/N rats were obtained from Lovelace Inhalation Toxicology Research Institute (Albuquerque, NM). Male and female B6C3F₁ mice were obtained from Simonsen Laboratory (Gilroy, CA). Rats and mice were held 3 weeks before the studies began, and were 6 to 7 weeks old when the studies began. Animal health was monitored by serologic analyses during the studies under the protocols of the NTP Sentinel Animal Program.

Animal Maintenance

Rats and mice were housed individually throughout the studies. Drinking water was available *ad libitum*. Further details of animal maintenance are given in Table K1.

Clinical Examinations and Pathology

All rats and mice were observed twice daily. Clinical observations and body weights were recorded at the beginning of the studies, each week, and at the end of the studies. Organ weights were recorded for the heart, right kidney, liver, and lung at the end of the studies.

A necropsy was performed on all animals. During necropsy, all organs and tissues were examined for grossly visible lesions. A complete histopathologic examination was performed on all high-exposure and control animals. Tissues for microscopic examination were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned to a thickness of 5 μ m, and stained with hematoxylin and eosin.

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4-Week Inhalation Studies

K-3

TABLE K1
Experimental Design and Materials and Methods in the 4-Week Inhalation Studies of Talc

Study Laboratory

Lovelace Inhalation Toxicology Research Institute (Albuquerque, NM)

Strain and Species

Rats: F344/N rats

Mice: B6C3F₁ mice

Animal Source

Rats: Lovelace Inhalation Toxicology Research Institute (Albuquerque, NM)

Mice: Simonsen Laboratory (Gilroy, CA)

Time Held Before Studies

3 weeks

Average Age When Placed on Studies

6-7 weeks

Date of First Exposure

Rats: 20 April 1983

Mice: 16 June 1983

Duration of Exposure

6 hours/day, 5 days/week for 4 weeks

Date of Last Exposure

Rats: 18 May 1983

Mice: 13 July 1983

Average Age When Killed

10 to 11 weeks

Method of Sacrifice

Intraperitoneal injection of T-61 solution

Necropsy Dates

Rats: 19-20 May 1983

Mice: 14-15 July 1983

Size of Study Groups

10 males and 10 females

Method of Animal Distribution

Randomized by weight

Animals per Cage

1

Method of Animal Identification

Ear tag and toeclip

Diet

NIH-07 Rat and Mouse Ration (Zeigler, Bros., Gardner, PA) available *ad libitum* during non-exposure periods

Maximum Storage Time for Feed

Not available

Water

Automatic Watering System (Edstrom Industries, Waterford, WI), available *ad libitum*

Cages

Stainless steel mesh cages (Hazelton, Aberdeen, MD), changed once weekly

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Talc, NTP TR 421

TABLE K1

Experimental Design and Materials and Methods in the 4-Week Inhalation Studies of Talc (continued)

Chambers

Stainless steel multitiered whole-body exposure chambers (H2000 and H1000, Hazleton Systems, Aberdeen, MD) washed once weekly

Excreta Pan

Techboard untreated paper (Shepherd Specialties Paper, Inc., Kalamazoo, MI), changed twice a day

Filters

Room Air and Chamber Air High Efficiency Particulate Air (HEPA) Filter, MIL Spec MIL-F-51068C (Flanders, Washington, DC), changed as required

Animal Room Environment

Rats

Average temperature: 23° C

Relative humidity: 40.3%

Fluorescent light: not available

Room air changes: not available

Mice

Average temperature: 24° C

Relative humidity: 42%

Fluorescent light: not available

Room air changes: not available

Exposure Concentrations

0, 2, 6, and 18 mg/m³ by inhalation

Type and Frequency of Observation

Observed twice daily; body weights and clinical findings recorded at study initiation and weekly thereafter

Necropsy

Necropsy was performed on all animals.

Histopathology

Complete histopathologic examinations performed on all high-exposure and control animals. In addition to tissue masses, gross lesions, and associated lymph nodes, tissues examined included: larynx, lung, nasal turbinates, trachea, and tracheobronchial lymph nodes.

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4-Week Inhalation Studies

K-5

RESULTS

Rats

All rats survived to the end of the study and there were no clinical findings related to talc exposure. The mean body weights and final mean body weights of exposed male and female rats were similar to those of the controls.

There were no significant increases in any organ-weight-to-body-weight ratios in male or female rats. The talc lung burdens increased with talc exposure level; however, the ratio of lung burden to exposure concentration was somewhat higher at the 6 and 18 mg/m³ exposure levels. The increase in talc lung burden to exposure concentration may be because the maximum ability of the respiratory tract to clear particles was exceeded at the 6 and 18 mg/m³ exposure levels.

There was a minimal increase in the number of intra-alveolar macrophages in the lung of male and female rats exposed to 18 mg/m³. The lesion was diffuse in nature and in no instance were clusters of greater than three alveolar macrophages observed. The individual macrophages were slightly larger than normal and had cytoplasm which contained fine eosinophilic granules.

Mice

One male mouse exposed to 2 mg/m³ and one male mouse exposed to 6 mg/m³ died before the end of the study. The survival of exposed male and female mice was similar to that of the controls. The mean weights and final mean body weights of exposed male and female mice were similar to those of the controls. There were no clinical findings associated with exposure to talc aerosols.

There were no significant changes in any organ-weight-to-body-weight ratios in exposed male or female mice. Talc lung burdens increased with talc exposure level. However, the ratio of lung burden to exposure concentration was constant at all exposure levels. In contrast to rats, the maximum ability of the respiratory tract to clear particles was apparently not exceeded at the 18 mg/m³ level.

The only lesions related to inhalation of talc aerosols were observed in the lung of male and female mice exposed to 18 mg/m³. However, the changes were minimal and consisted of a diffuse increase in the number of intra-alveolar macrophages. In most cases, pulmonary macrophages did not exceed two per alveolus, but occasional clusters of up to 10 alveolar macrophages were observed. The individual macrophages were two to three times normal size with foamy granular cytoplasm.

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